Studies toward a prebiotic protometabolism

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UCL

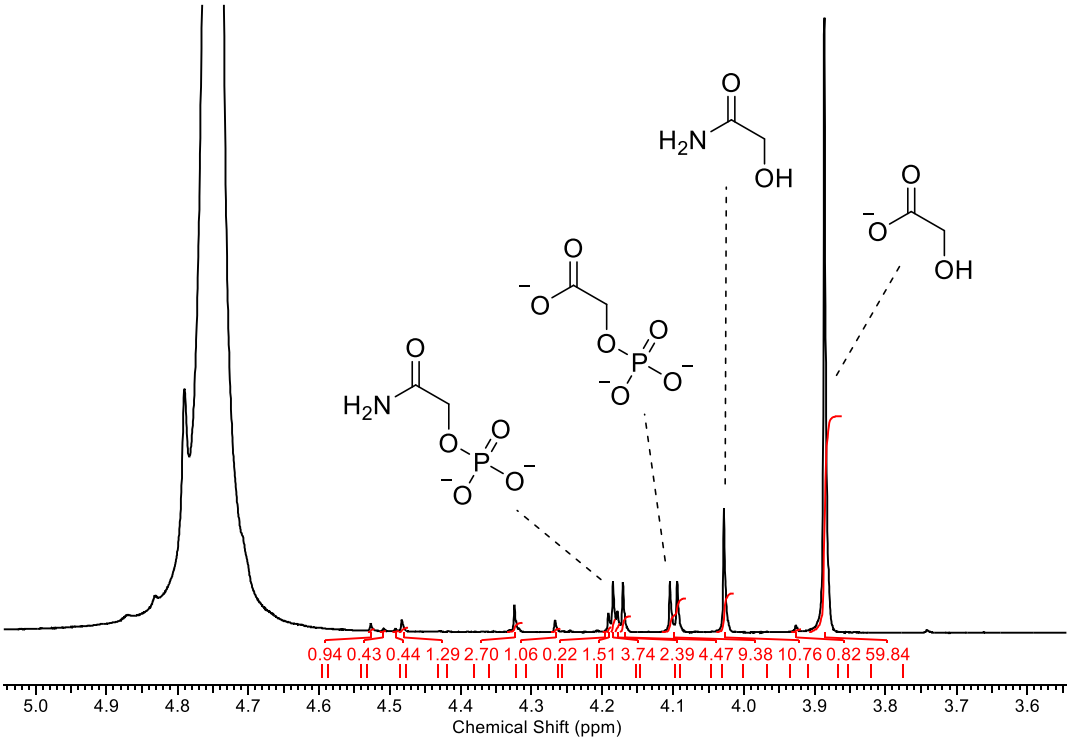
Thesis Submitted to University College London
for the Degree of Doctor of Philosophy

Appendix – part 1

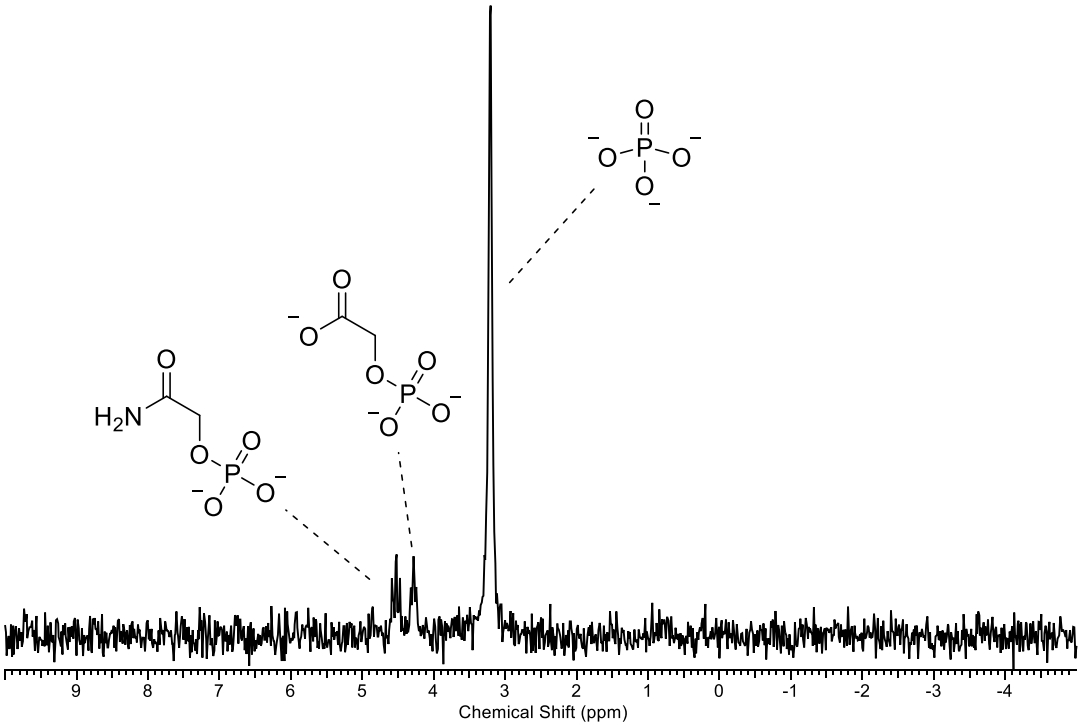
Triose Glycolysis

Urea-catalysed phosphorylations

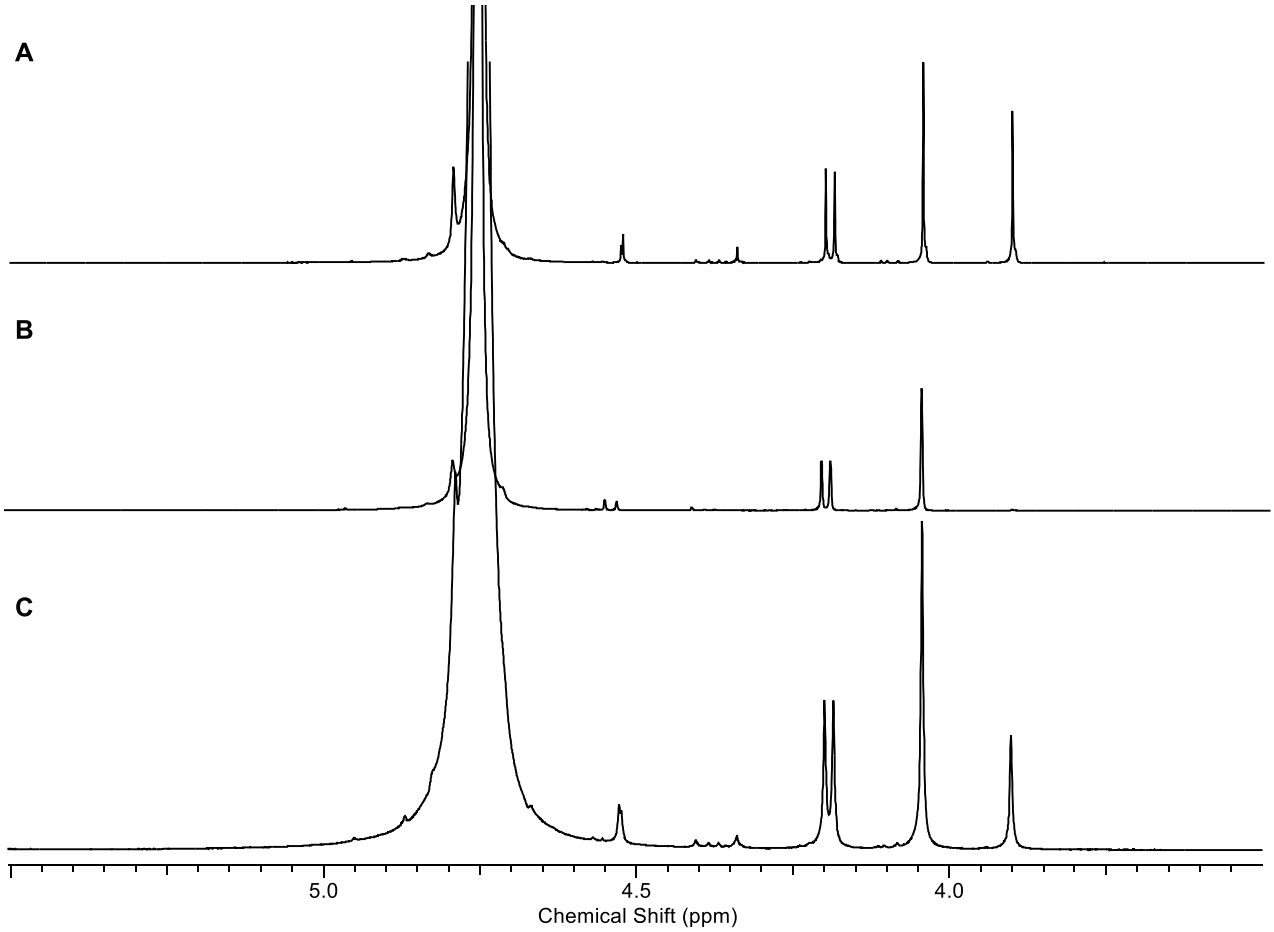
A1: ^{1}H NMR spectrum (500 MHz, {D_2O}, 3.5 – 5.0 ppm) of the reaction of glycolic acid (**45**, 0.24 mmol) with urea (2.4 mmol) and diammonium hydrogen phosphate (0.24 mmol) at 100 °C, 3 h (method A).



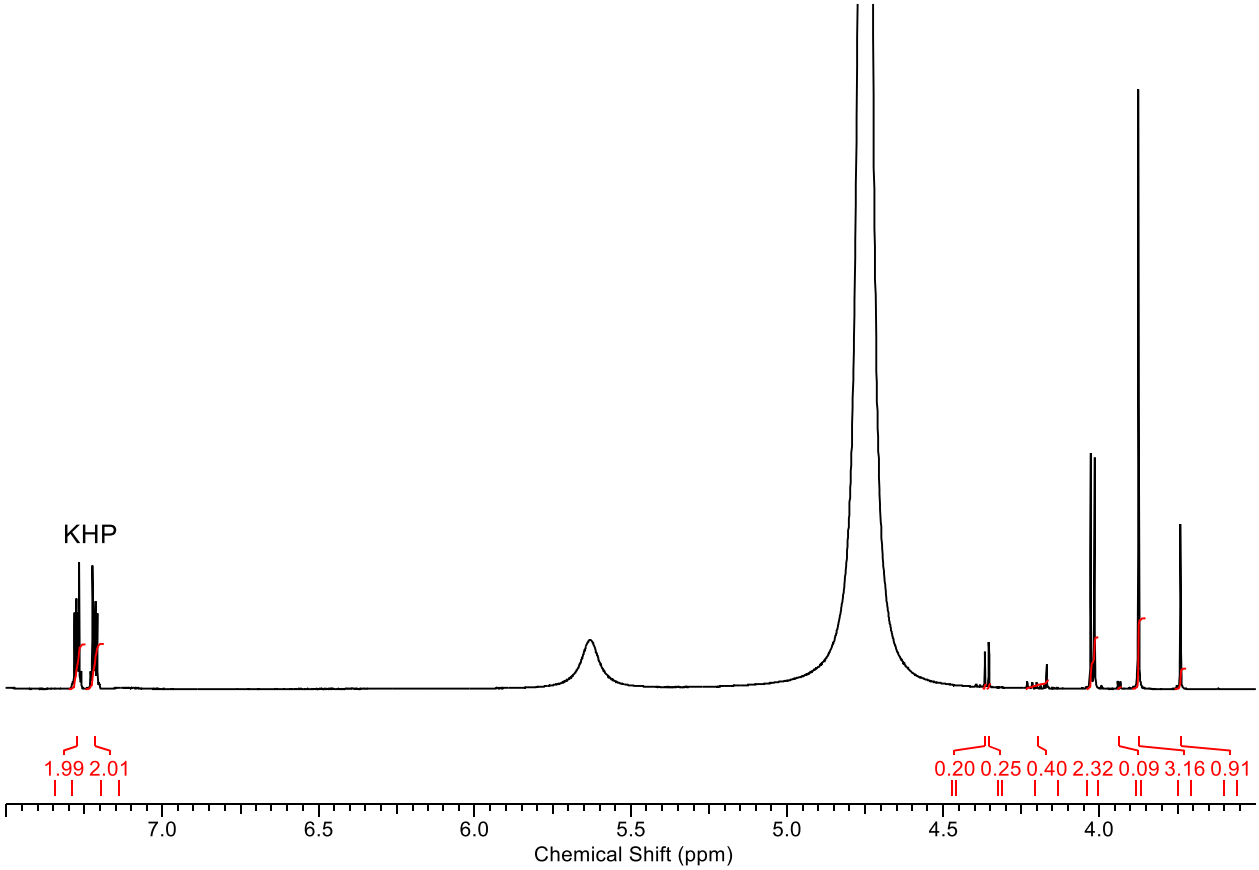
A2: ^{31}P NMR spectrum (121 MHz, {D_2O}, 3.5- 5.0 ppm) of the reaction of glycolic acid (**45**, 0.24 mmol) with urea (2.4 mmol) and diammonium hydrogen phosphate (0.24 mmol) at 100 °C, 3 h (method A).



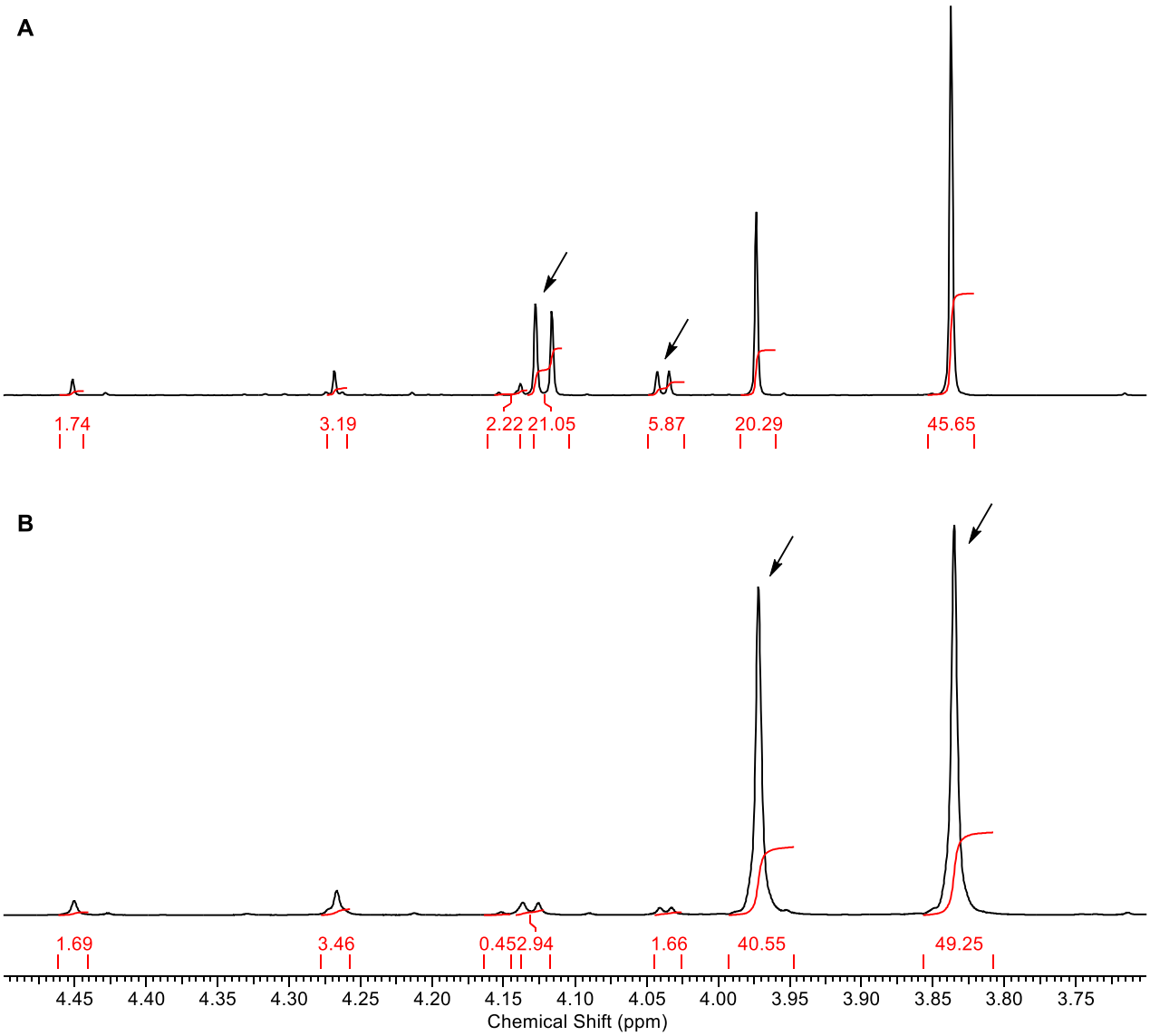
A3: ^{1}H NMR spectra (500 MHz, {D_2O}, 3.5 – 5.5 ppm) of the reactions with urea (2.4 mmol) and diammonium hydrogen phosphate (0.24 mmol) at 100 °C, 26 h (method A) of **A**, glycolic acid (**45**, 0.24 mmol), **B**, glycolamide (**102**, 0.24 mmol) and **C**, the two NMR samples combined, showing coincidental peaks.

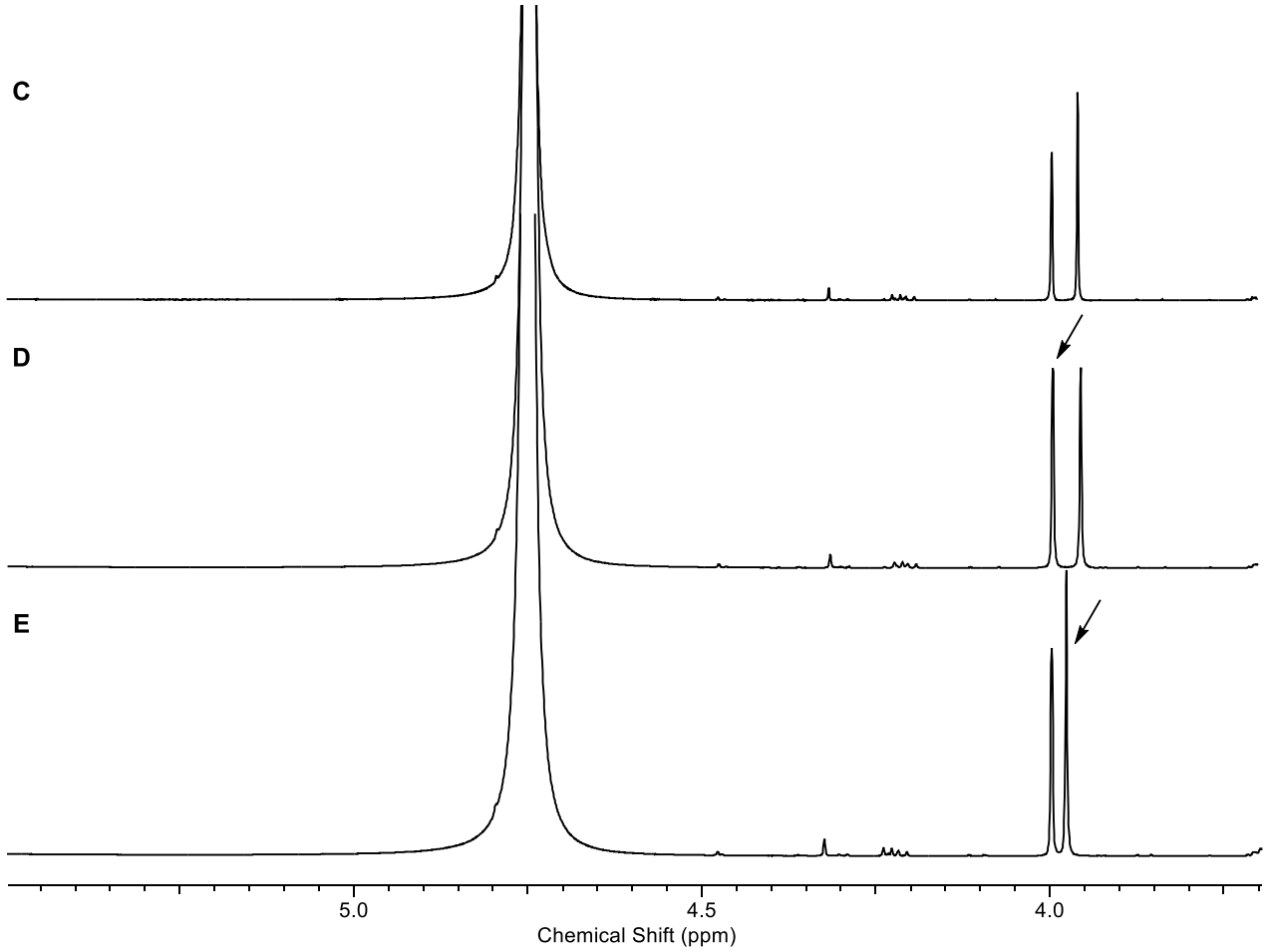


A4: ^{1}H NMR spectrum (600 MHz, {D_2O}, 3.5 – 7.5 ppm) of the reaction of glycolic acid (**45**, 0.24 mmol) with urea (2.4 mmol) and diammonium hydrogen phosphate (0.96 mmol) at 100 °C, 48 h (method B).



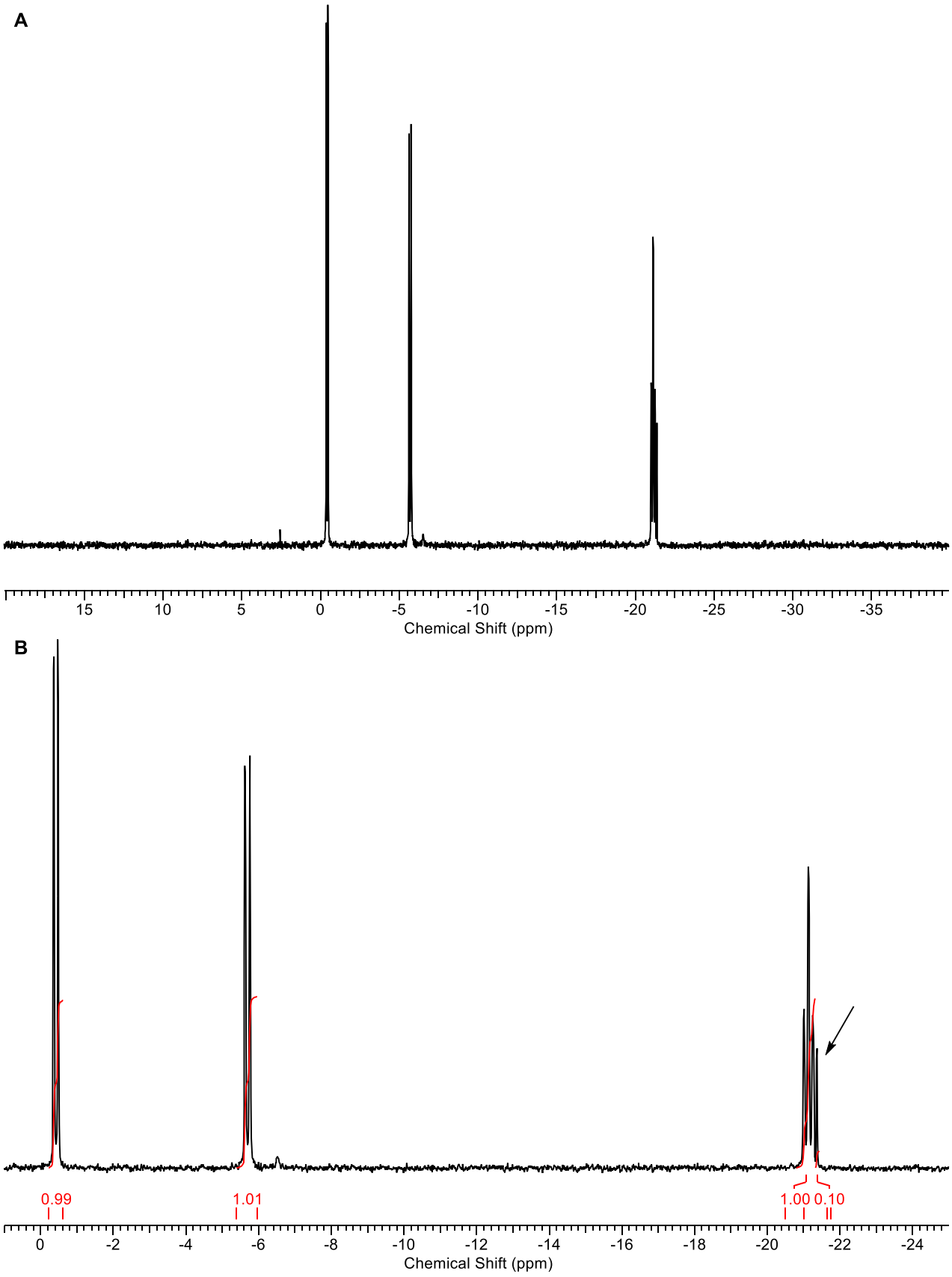
A5: **Top**, ^{1}H NMR spectra (600 MHz, {D_2O}, 3.7 – 4.5 ppm) of **A**, the reaction of glycolic acid (**45**, 0.24 mmol) with urea (2.4 mmol) and diammonium hydrogen phosphate (0.24 mmol) at 100 °C, 7 h and **B**, the same sample after incubation with phosphatase solution (method C). The diminishing peaks of glycolamide phosphate (**103**) and glycolic acid phosphate (**46**) and the intensifying peaks of glycolamide (**102**) and glycolic acid (**45**) are indicated with arrows. **Bottom**, ^{1}H NMR spectra (600 MHz, {D_2O}, 3.7 – 5.5 ppm) of **C**, the phosphatase reaction after lyophilising and re-dissolving (the change in chemical shift is due to a drop in pD, most likely due to loss of residual ammonia) and then after spiking with **D**, **102** and **E**, **45**. Positive spikes are indicated with arrows.




C
D
E
5.0
4.5
4.0
Chemical Shift (ppm)

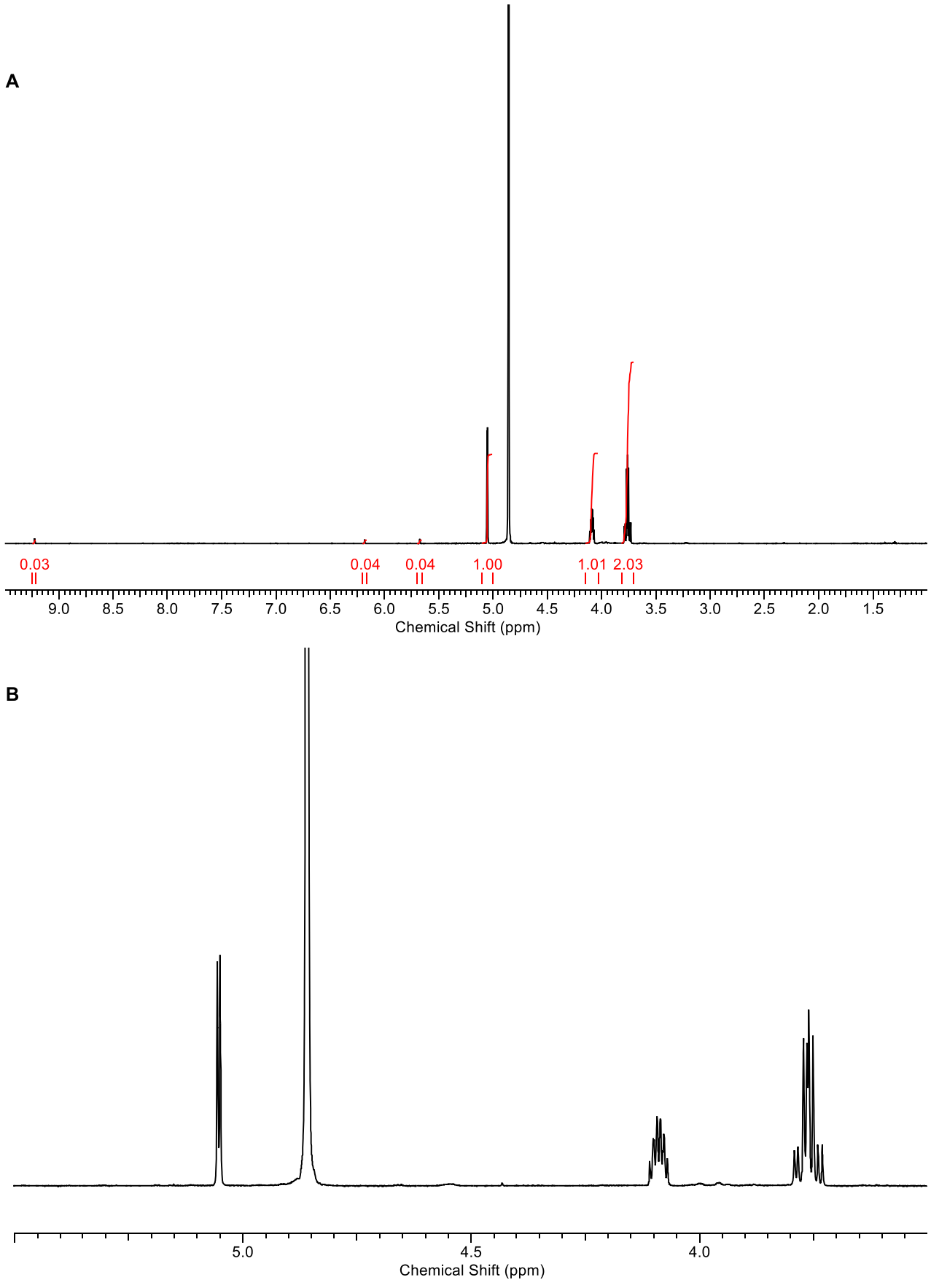
Amidotriphosphate (**47**)

A6: ^{31}P NMR spectra (121 MHz, {D_2O}, **A**, -40.0 – 20.0 ppm and **B**, -25.0 – 1.0 ppm) of aqueous **47** solution (~1M). Residual starting material (~3%) is indicated.

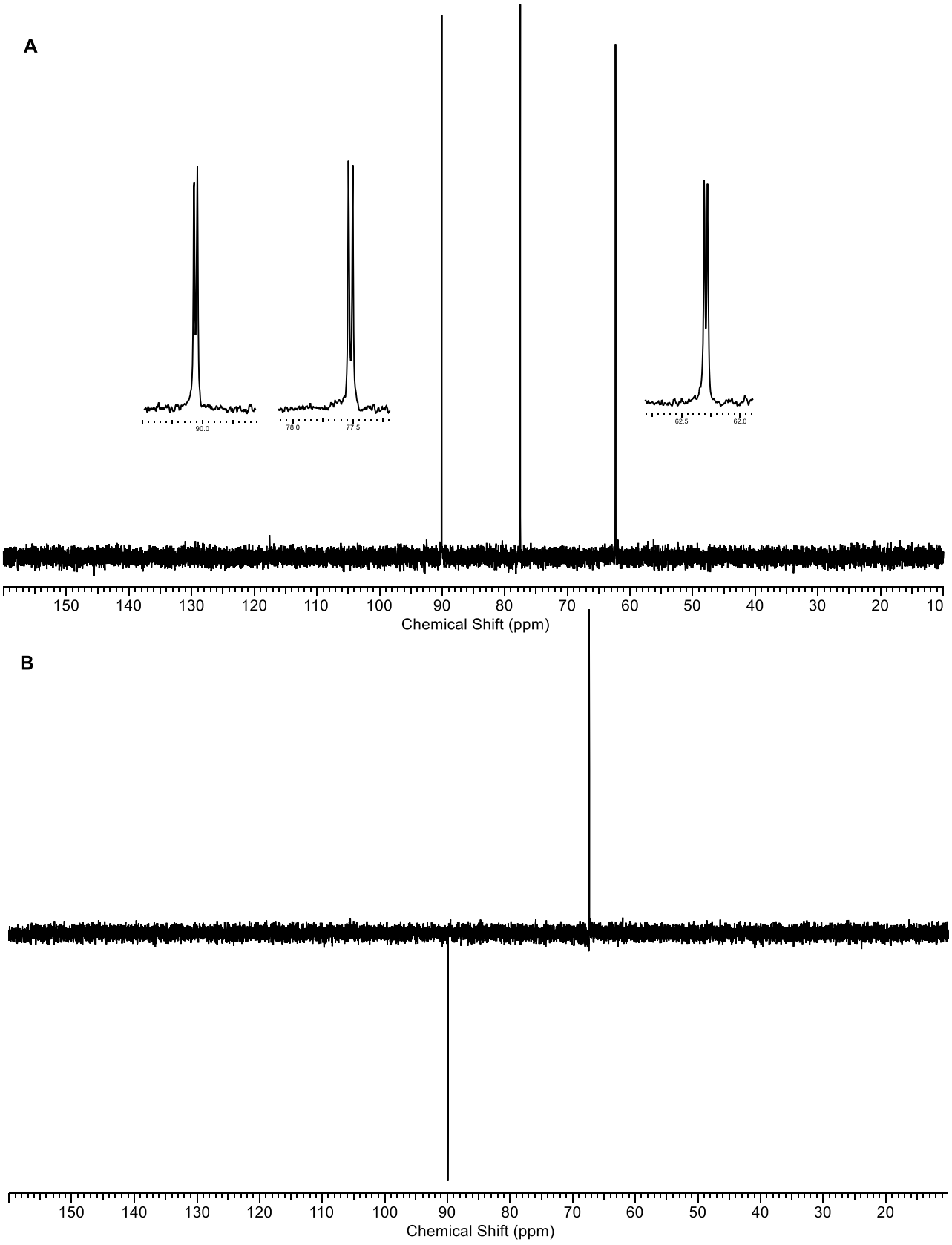


Glyceraldehyde-2-phosphate (**29**)

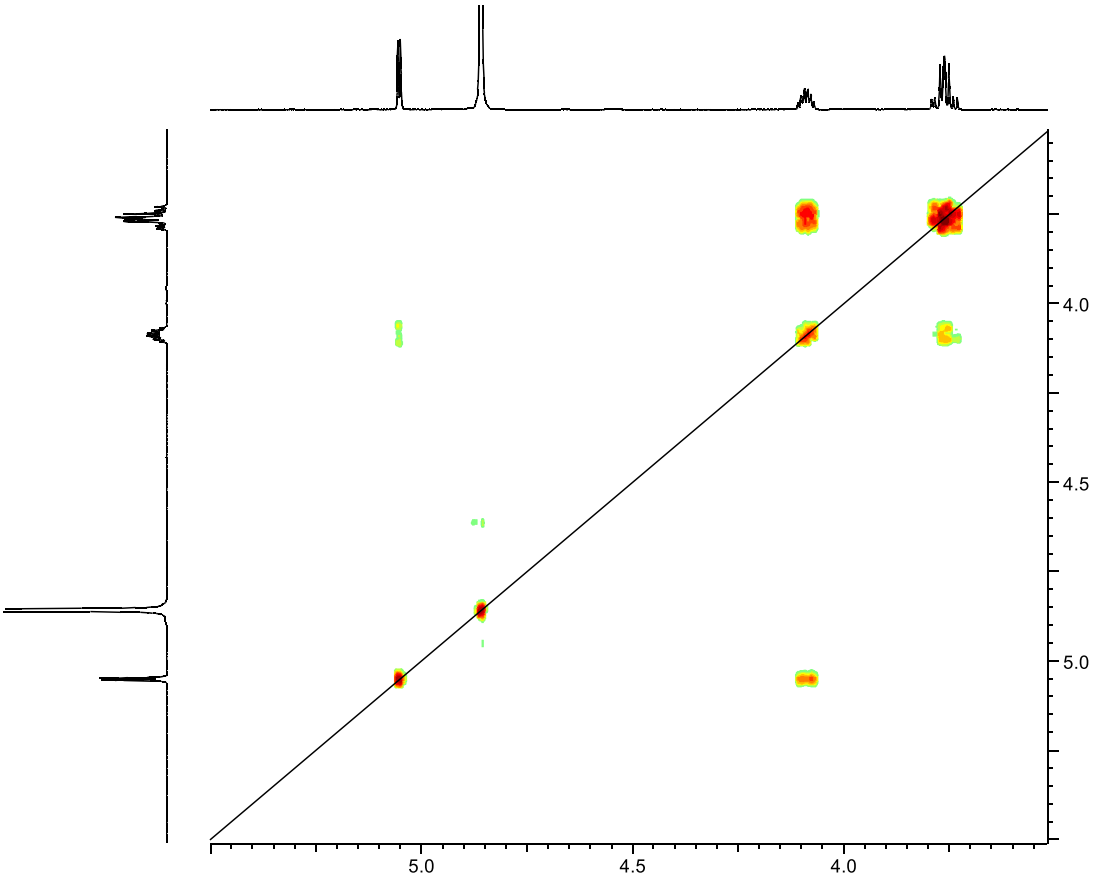
A7: ^{1}H NMR spectra (600 MHz, {D2O}, **A**) 1.0 – 9.5 ppm and **B**) 3.5 – 5.5 ppm) of **29** sodium salt.



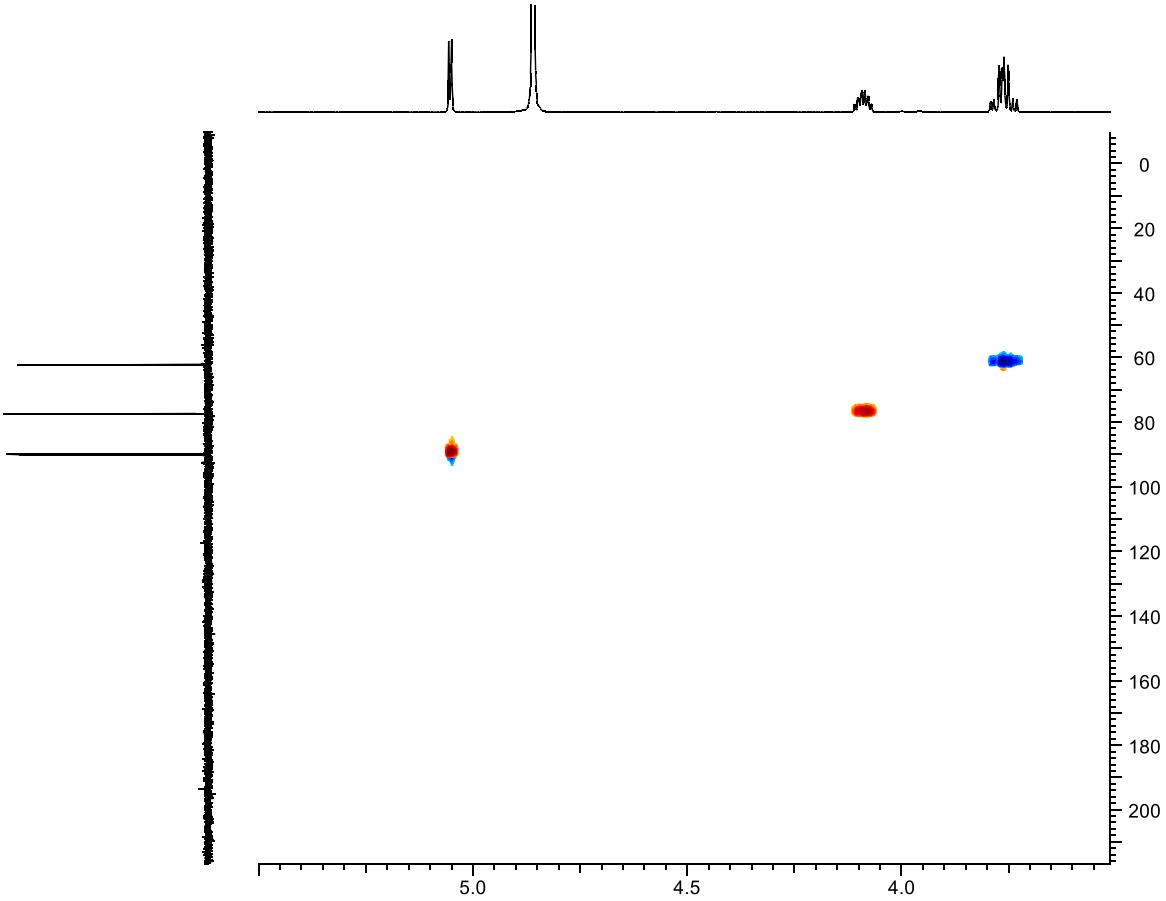
A8: **A**, ^{13}C and **B**, DEPT-135 NMR spectra (151 MHz, {D2O} 10–160 ppm) of **29** sodium salt.



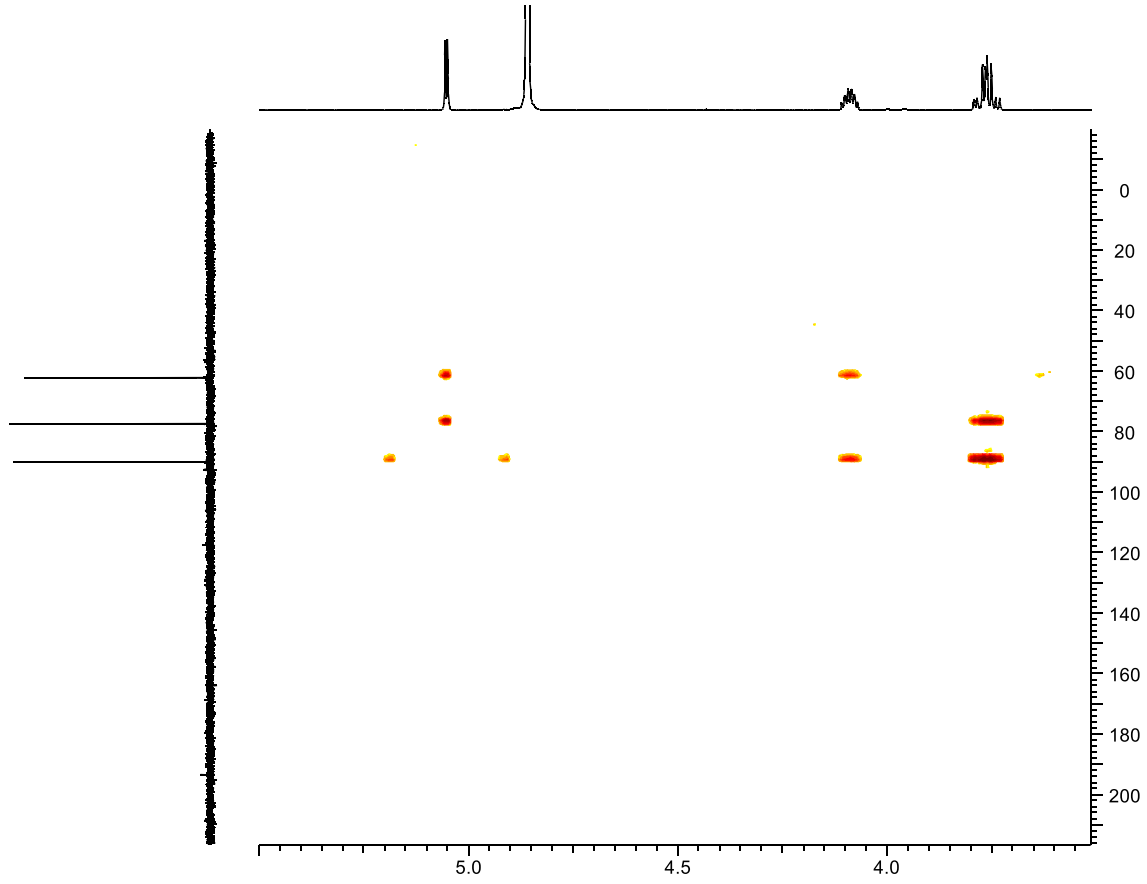
A9: ^{1}H-^{1}H COSY NMR spectrum (600 MHz, {D2O} 3.5 – 5.5 ppm), of **29** sodium salt.



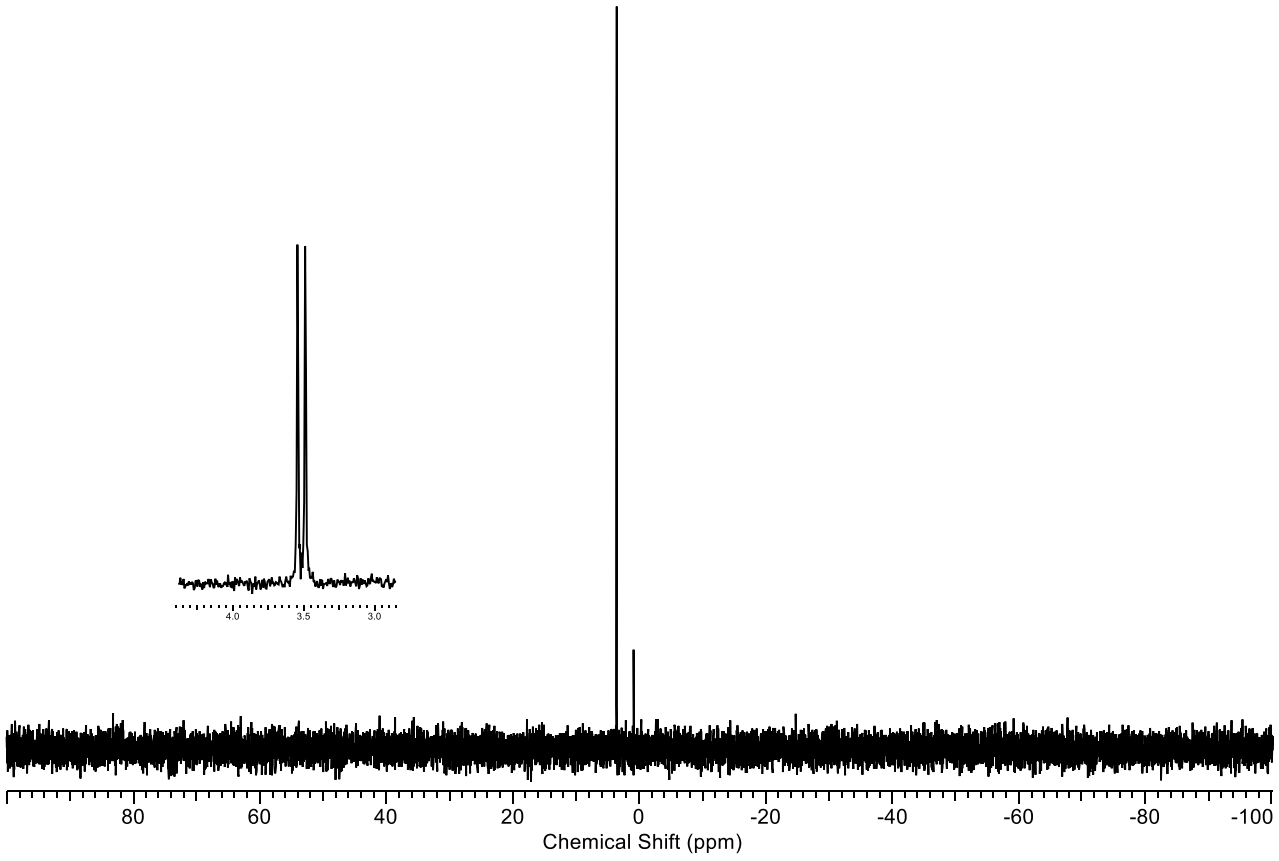
A10: ^{1}H-^{13}C HSQC NMR spectrum (600 MHz, {D_2O}) of **29** sodium salt.



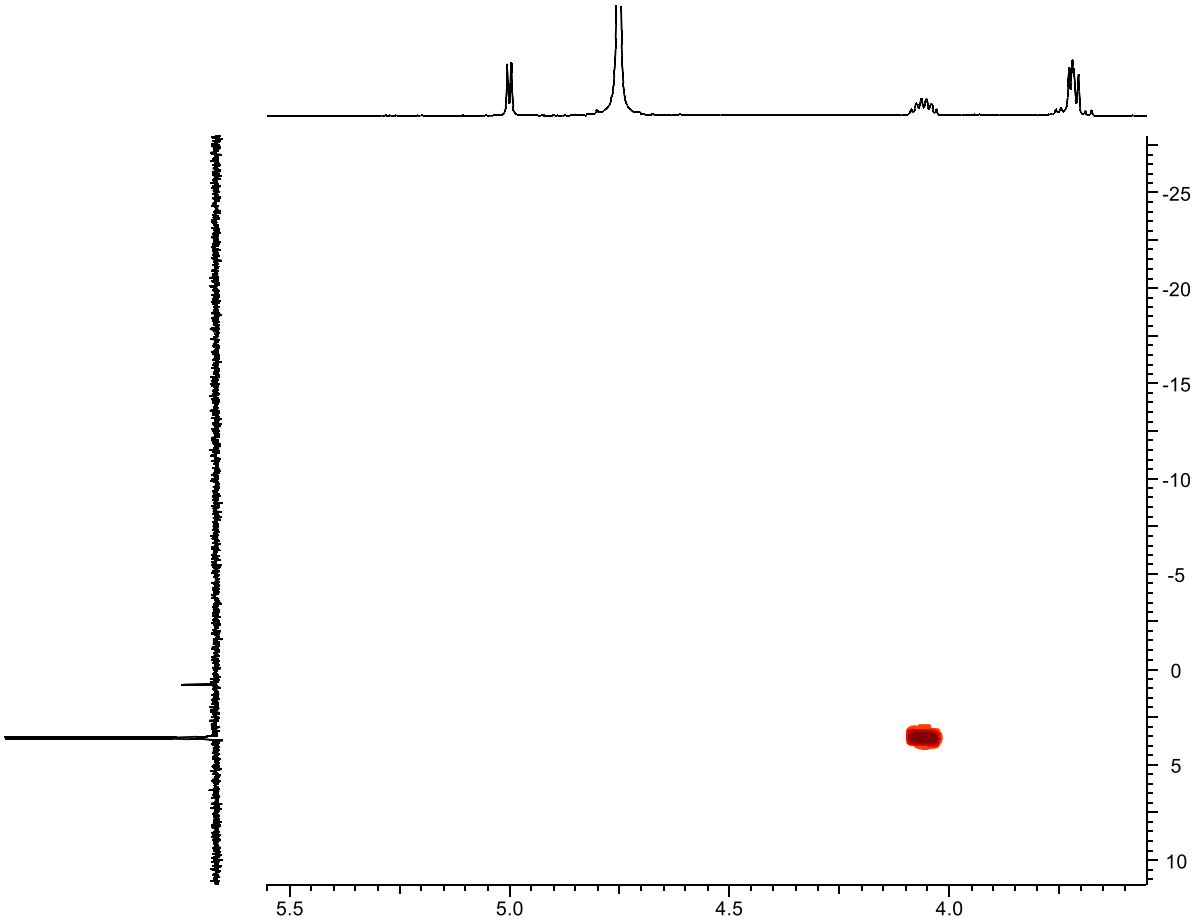
A11: ^{1}H-^{13}C HMBC NMR spectrum (600 MHz, {D_2O}) of **29** sodium salt.



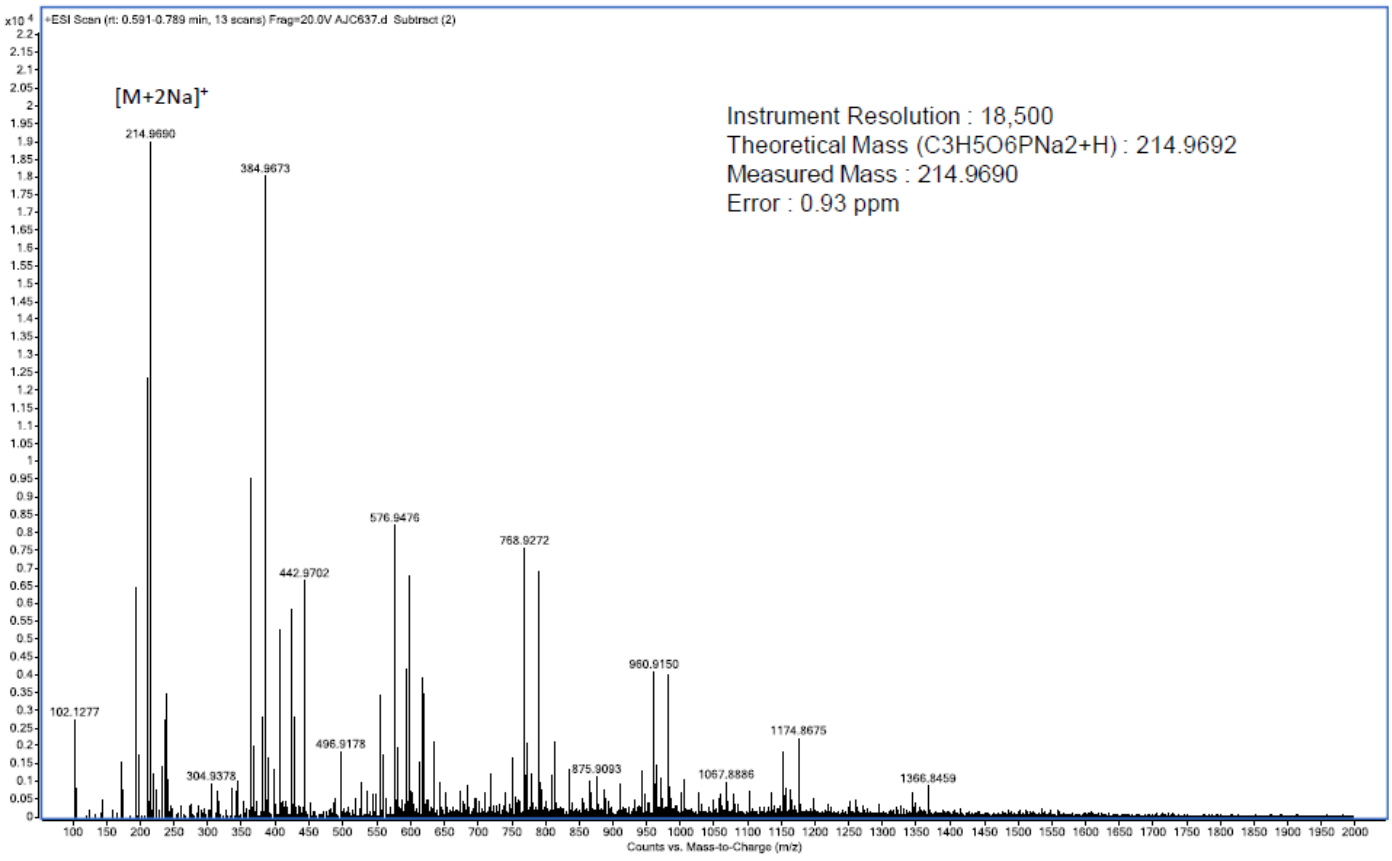
A12: ^{31}P NMR spectrum (161 MHz, {D_2O} -100 – 100 ppm) of **29** sodium salt with $^{31}P\{^{1}H$-coupled} signal overlaid.



A13: ^{1}H-^{31}P HMBC NMR spectrum (400 MHz, {D_2O}) of **29** sodium salt.

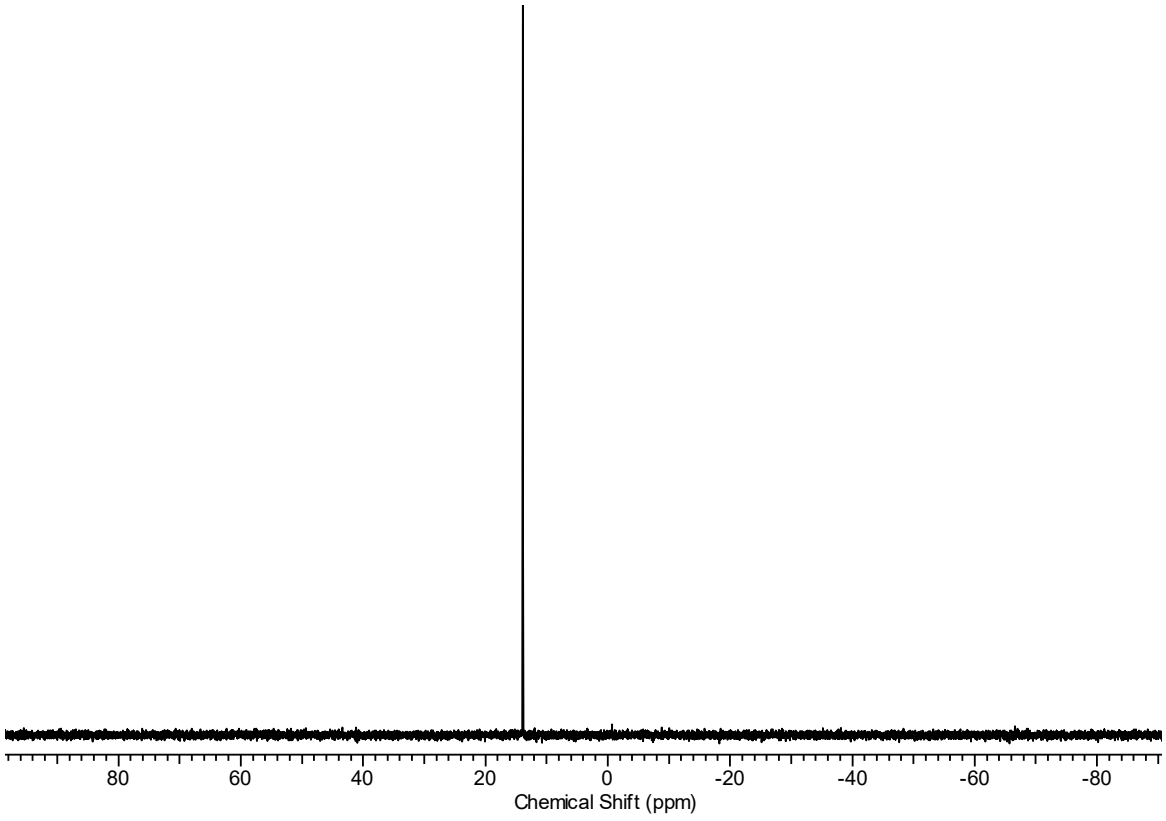


A14: ESI+ mass spectrum of **29** sodium salt.

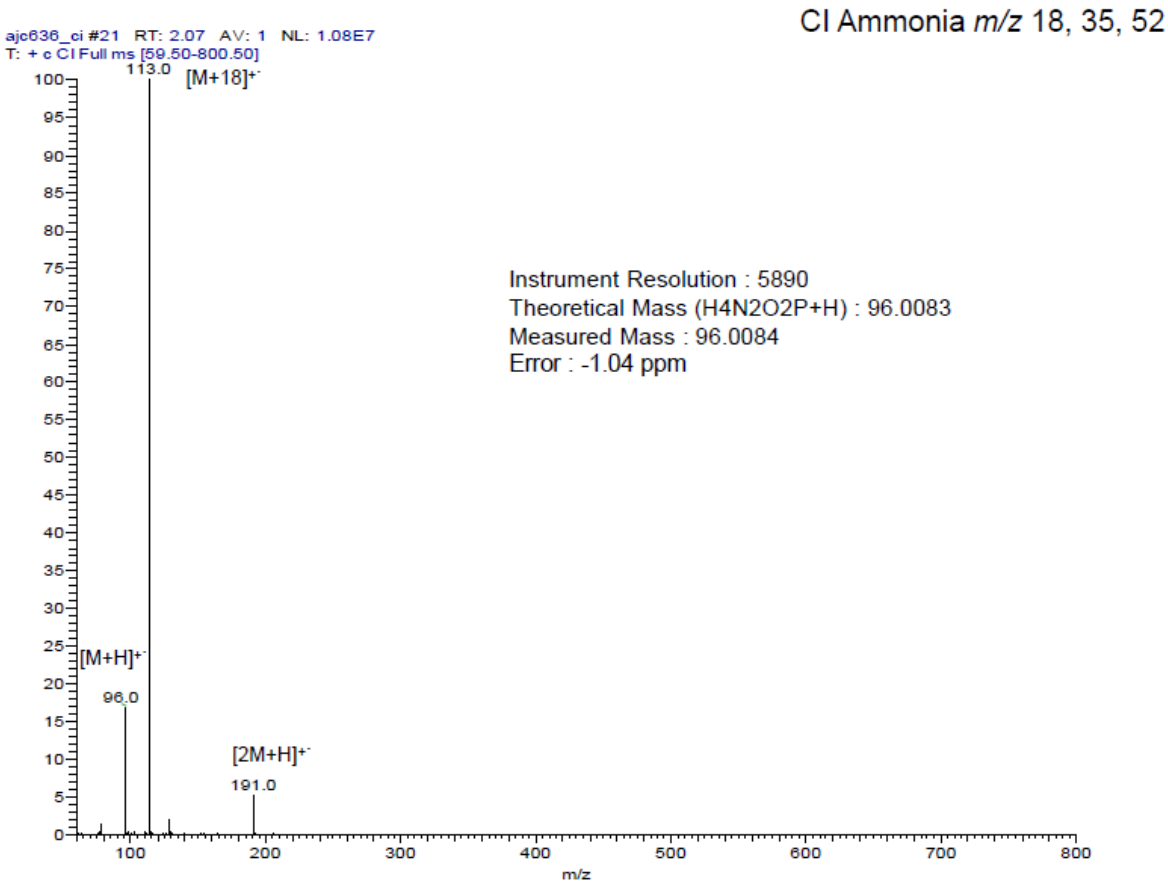


Diamidophosphate (**48**)

A15: ^{31}P NMR spectrum (161 MHz, {D_2O} -100 – 100 ppm, pD 9.9) of **48** sodium salt.

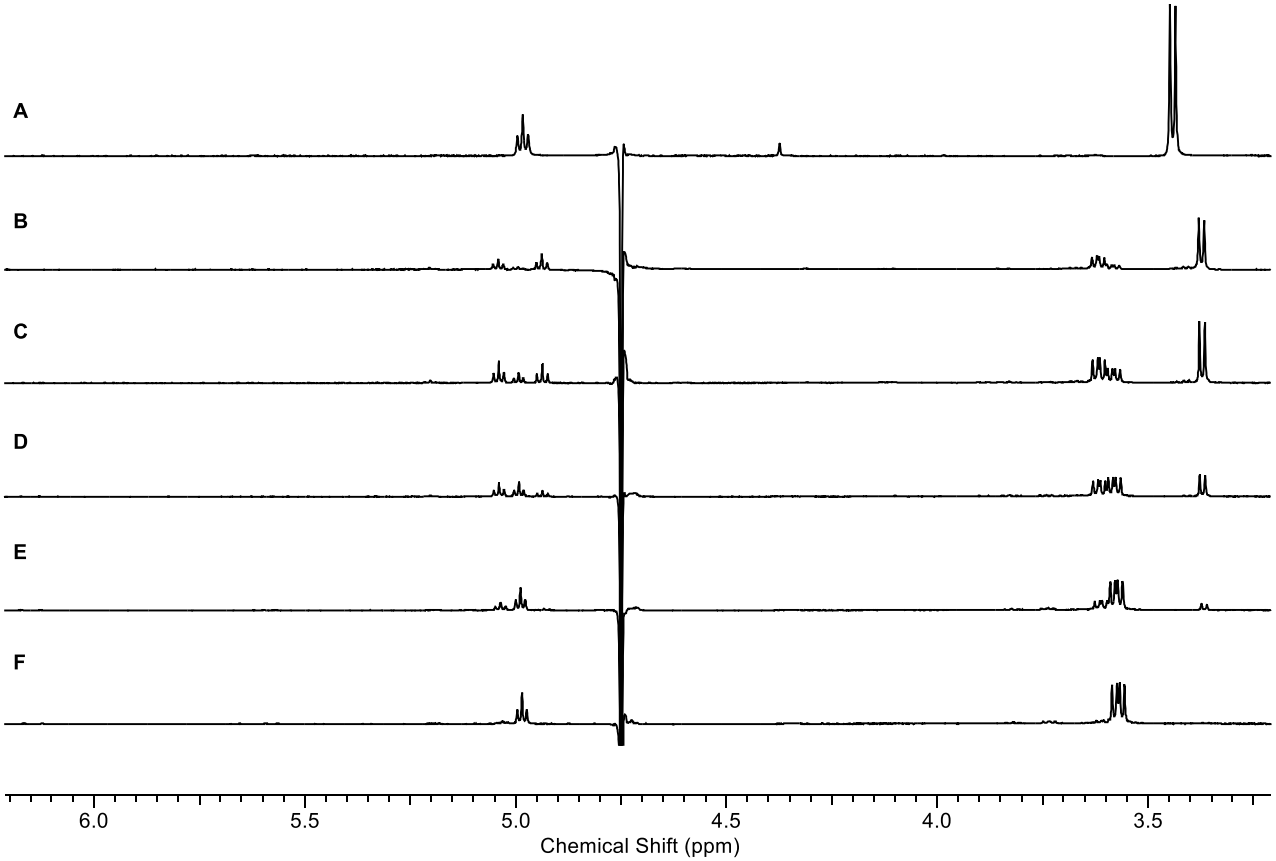


A16: CI+ mass spectrum of **48** sodium salt.

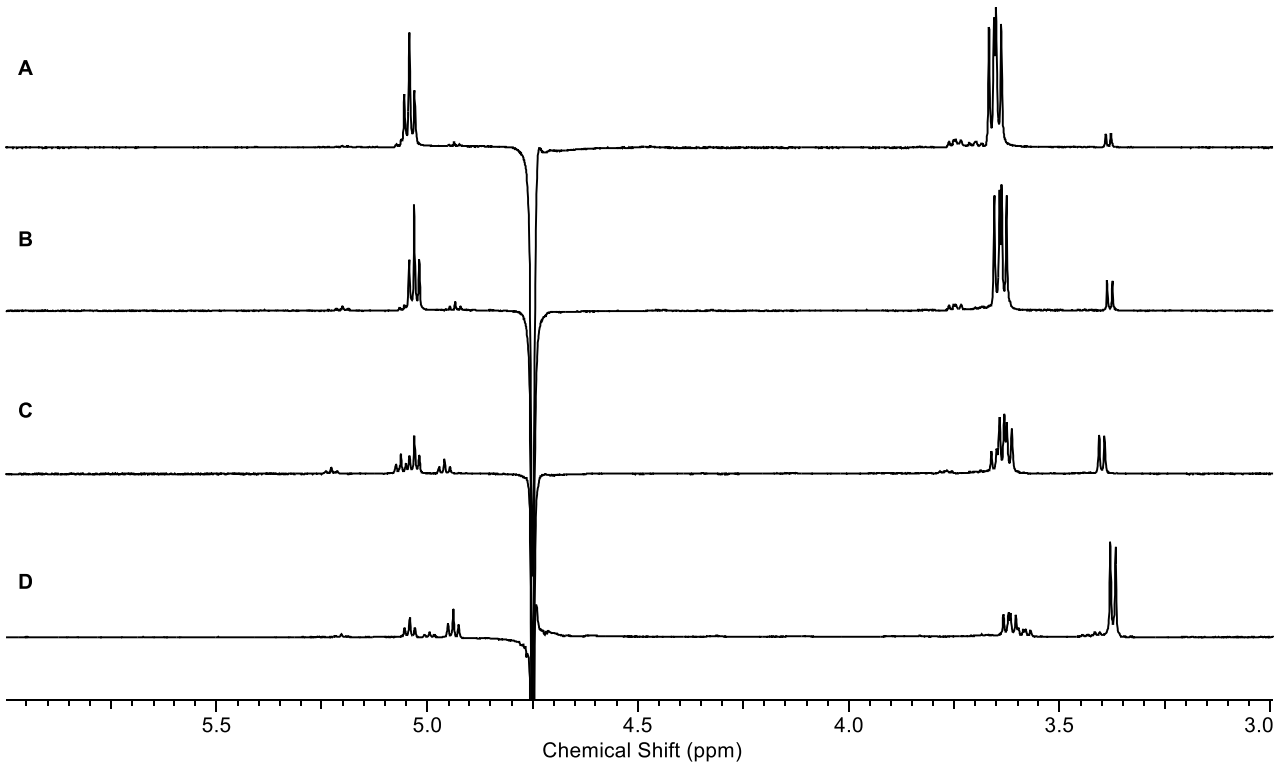


Diamidophosphate (**48**) phosphorylations

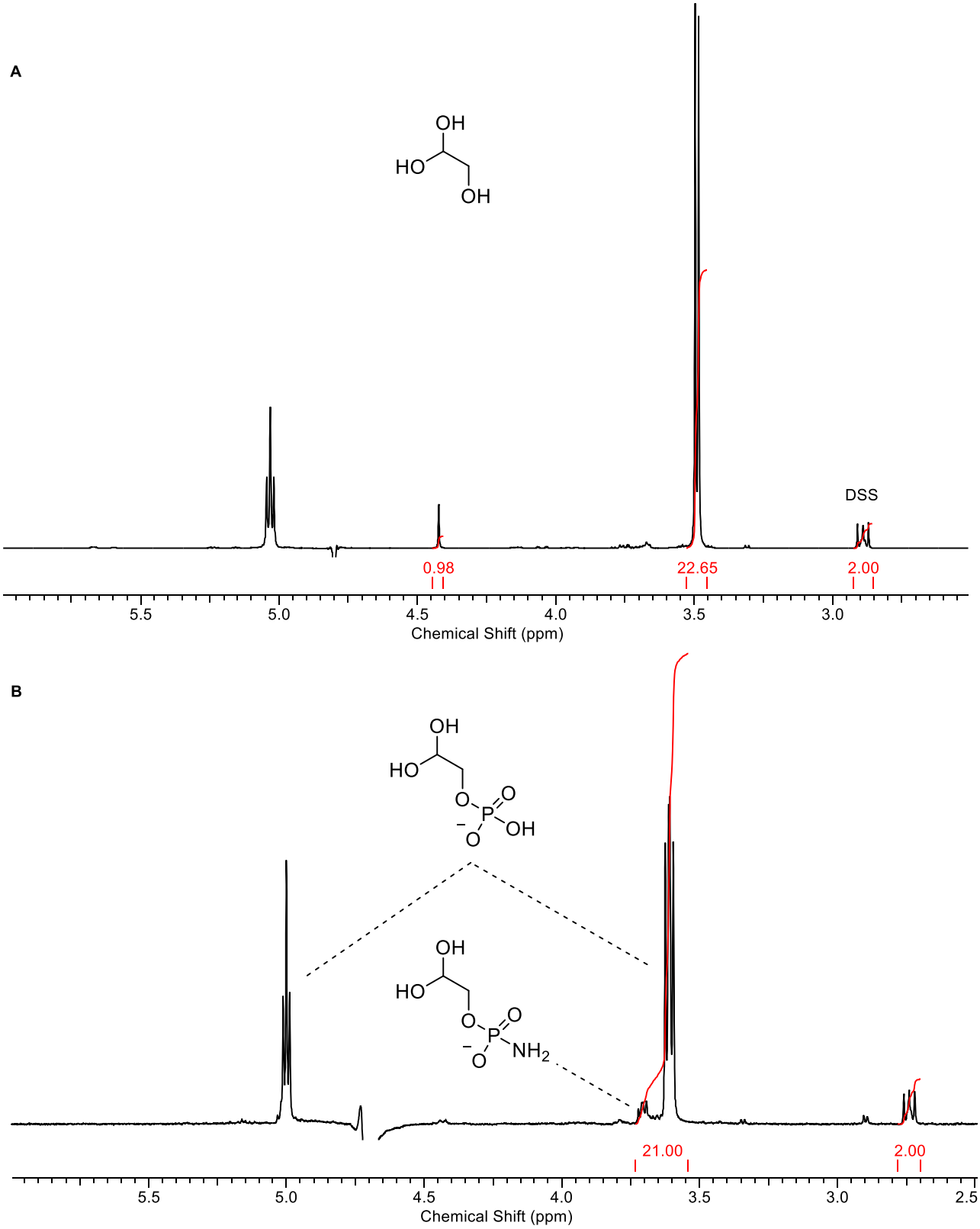
A17: ^{1}H NMR spectra (400 MHz, {750mM phosphate H_2O/D_2O 9:1}, 3.2 – 6.2 ppm) of glycolaldehyde (**26**, 25mM) and diamidophosphate (**48**, 100mM) with DSS, at room temperature, pH 7. **A**, 0.0 h, **B**, 1.5 h, **C**, 2.5 h, **D**, 4.5 h, **E**, 8.5 h, **F**, 22 h.



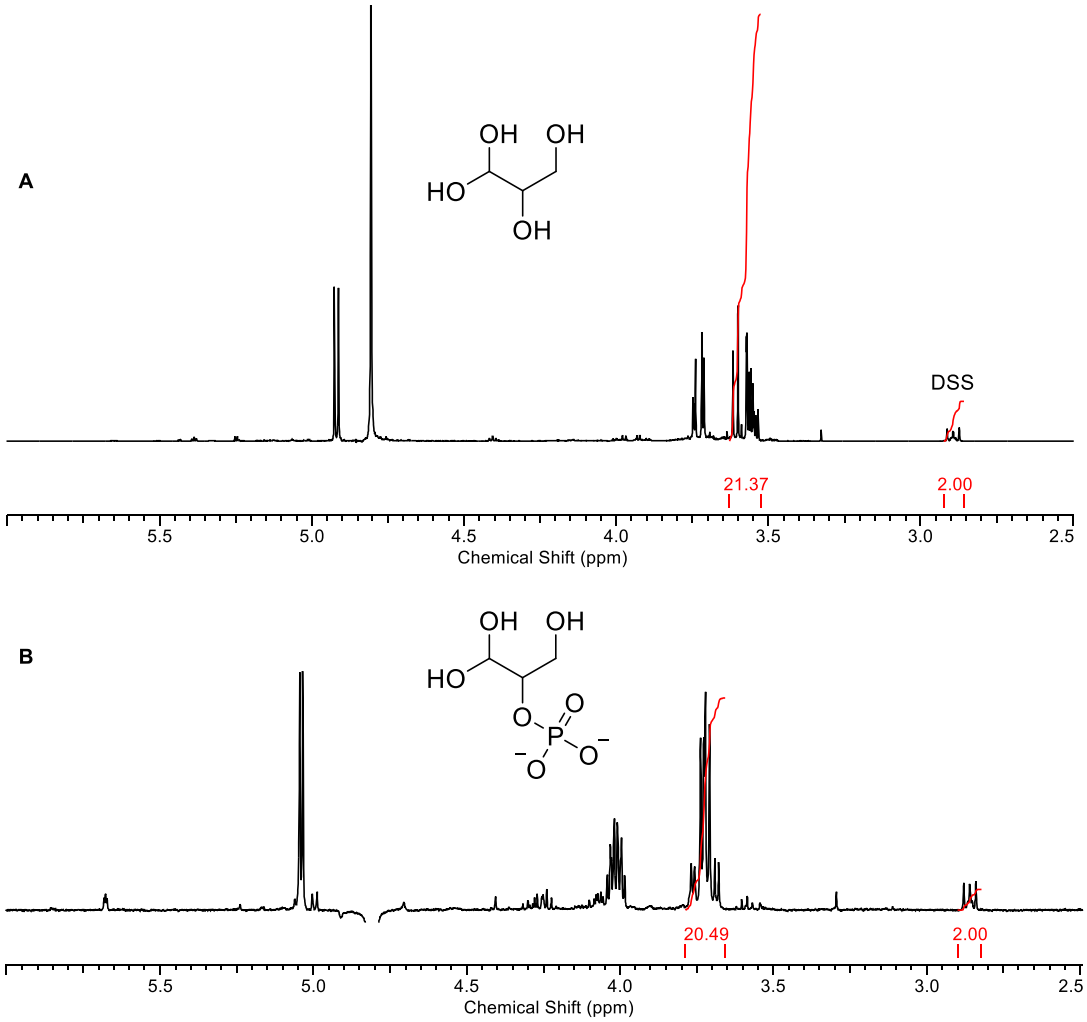
A18: ^{1}H NMR spectra (400 MHz, {750mM phosphate, H_2O/D_2O, 9:1}, 3.0 – 6.0 ppm) of glycolaldehyde (**26**, 25mM) and diamidophosphate (**48**, 100mM) after 1.6 h at room temperature, at: **A**, pH 4, **B**, pH 5, **C**, pH 6 and **D**, pH 7.



A19: ^{1}H NMR spectra (400 MHz, {750mM phosphate H_2O/D_2O 9:1}, 2.5 – 6.0 ppm) of: **A**, glycolaldehyde (**26**, 25mM) with DSS and **B**, after incubation with diamidophosphate (**48**, 100mM) at room temperature, pH 4, 4 h.

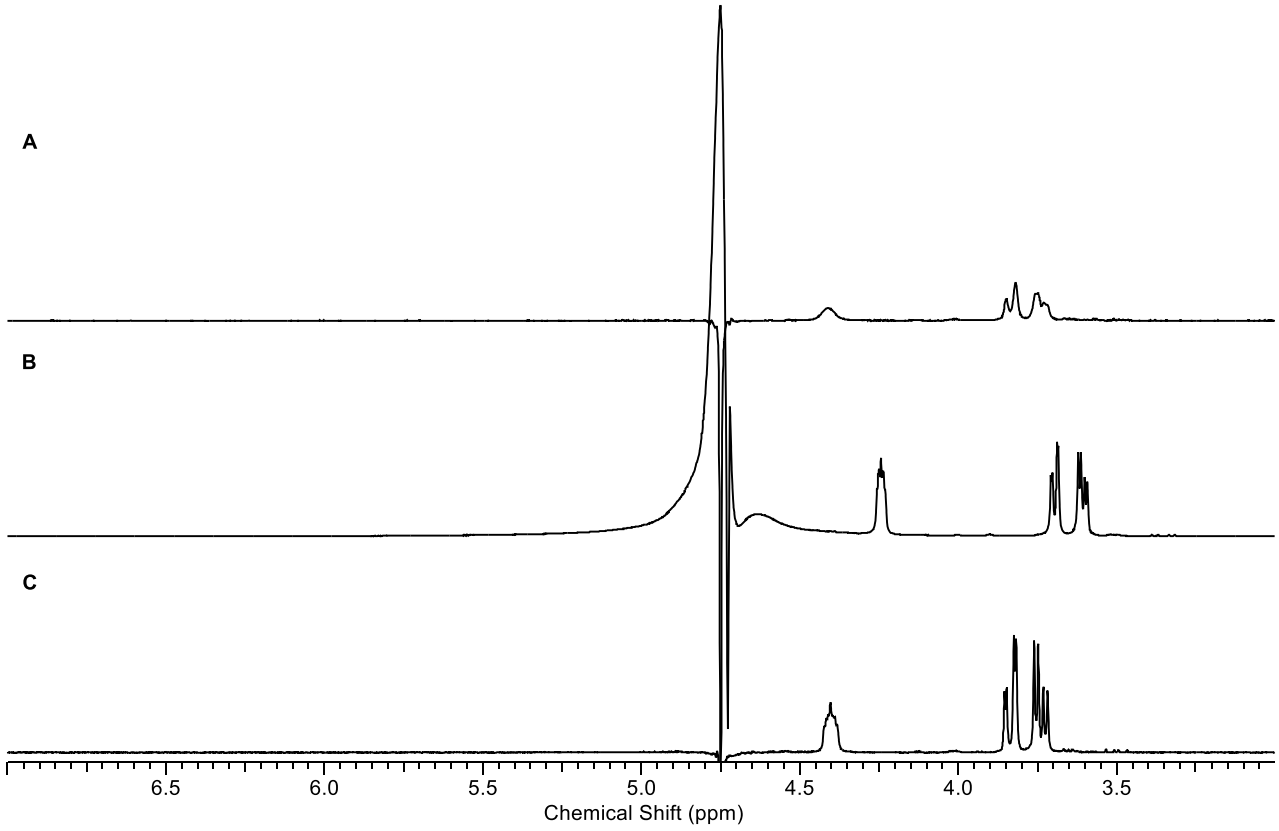


A20: ^{1}H NMR spectra (400 MHz, {750mM phosphate H_2O/D_2O, 9:1}, 2.5–6.0 ppm) of **A**, glyceraldehyde (**27**, 25mM) with DSS and **B**, after incubation with diamidophosphate (**48**, 100mM) at room temperature, pH 4, 4 h.



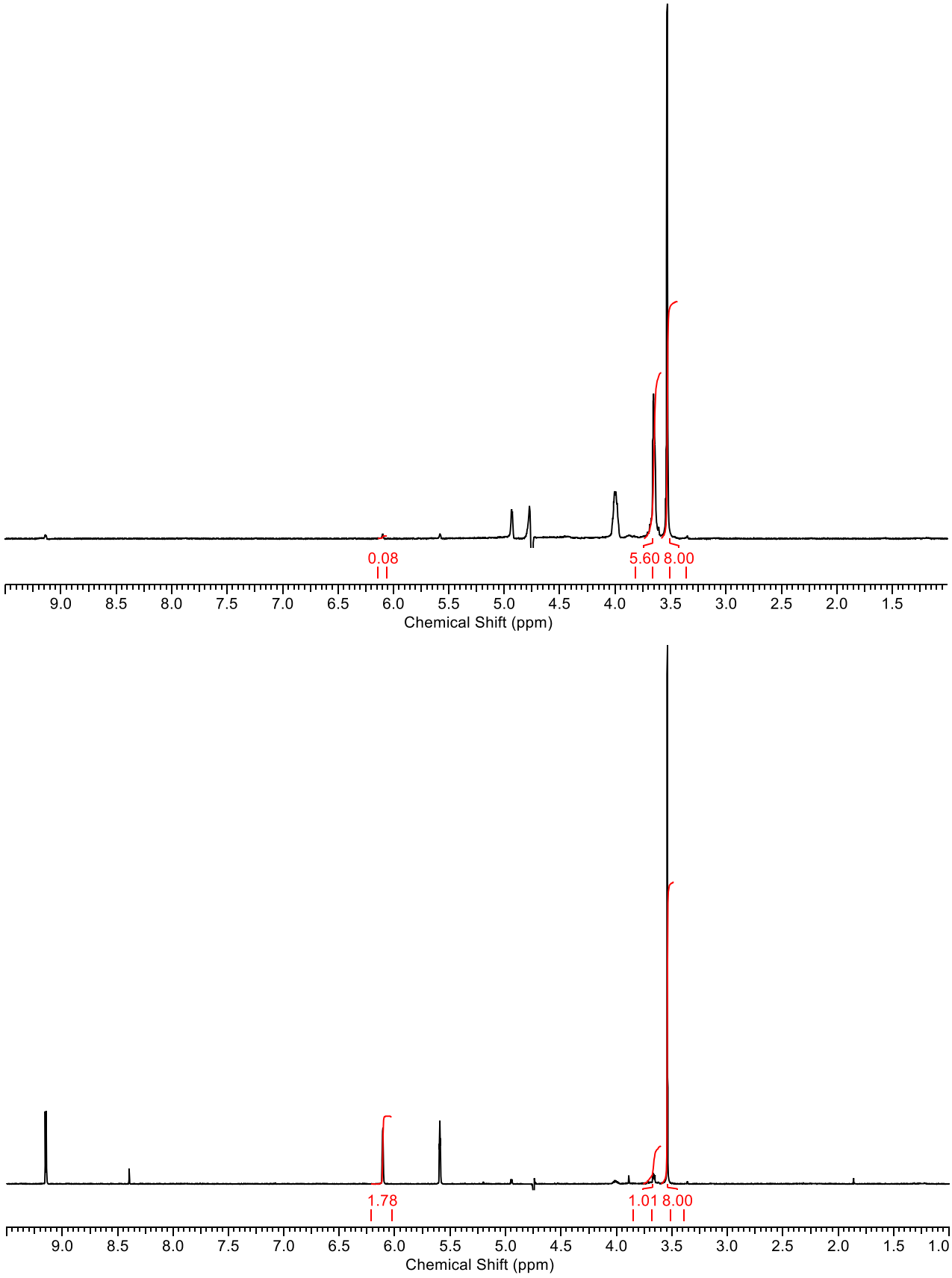
Dehydration experiments

A21: ^{1}H NMR spectra (400 MHz, {H_2O/D_2O 9:1}, 3.0–7.0 ppm, pH 7)[i] of glycerate-2-phosphate (**43**, 60 – 100mM) incubated at 60 ºC for 15 h at **A**, pH 2 **B,** pH 7 (1M phosphate buffer) and **C**, pH 10.

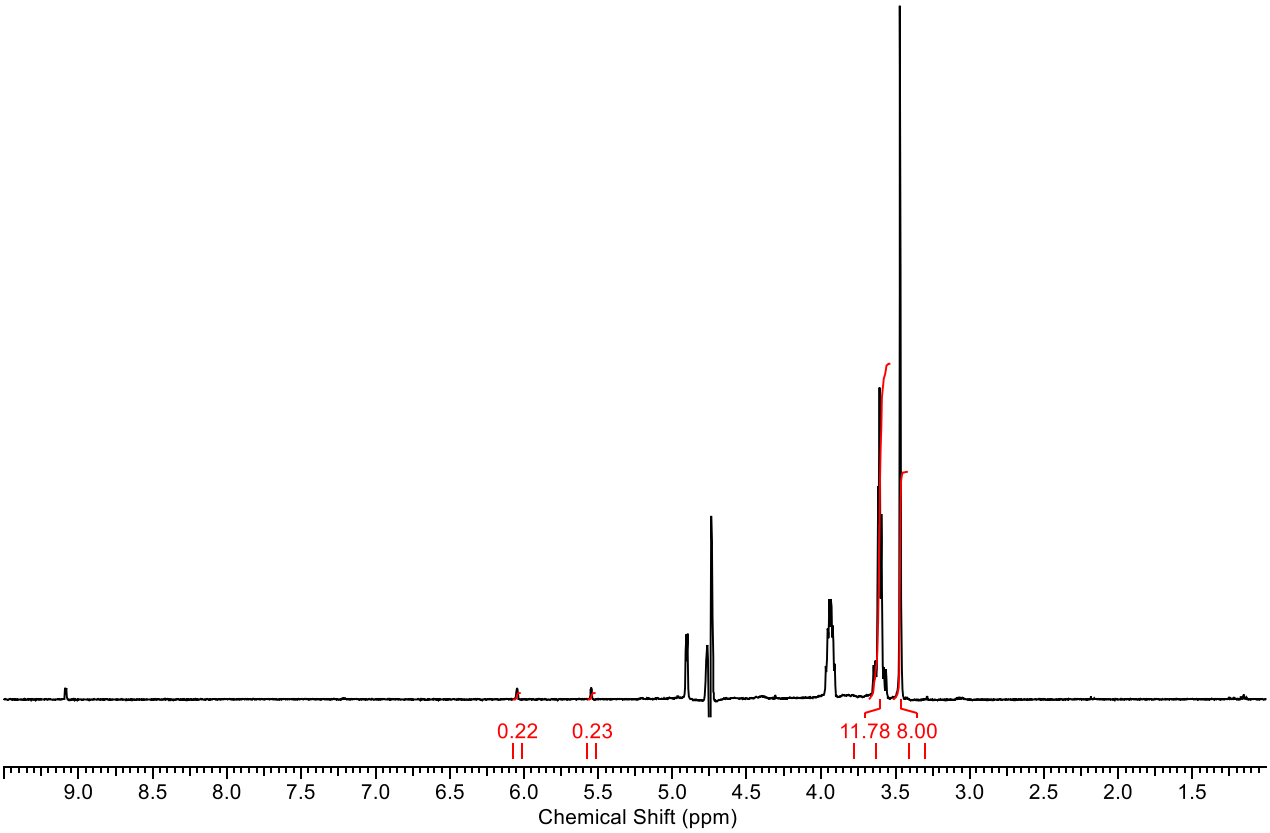


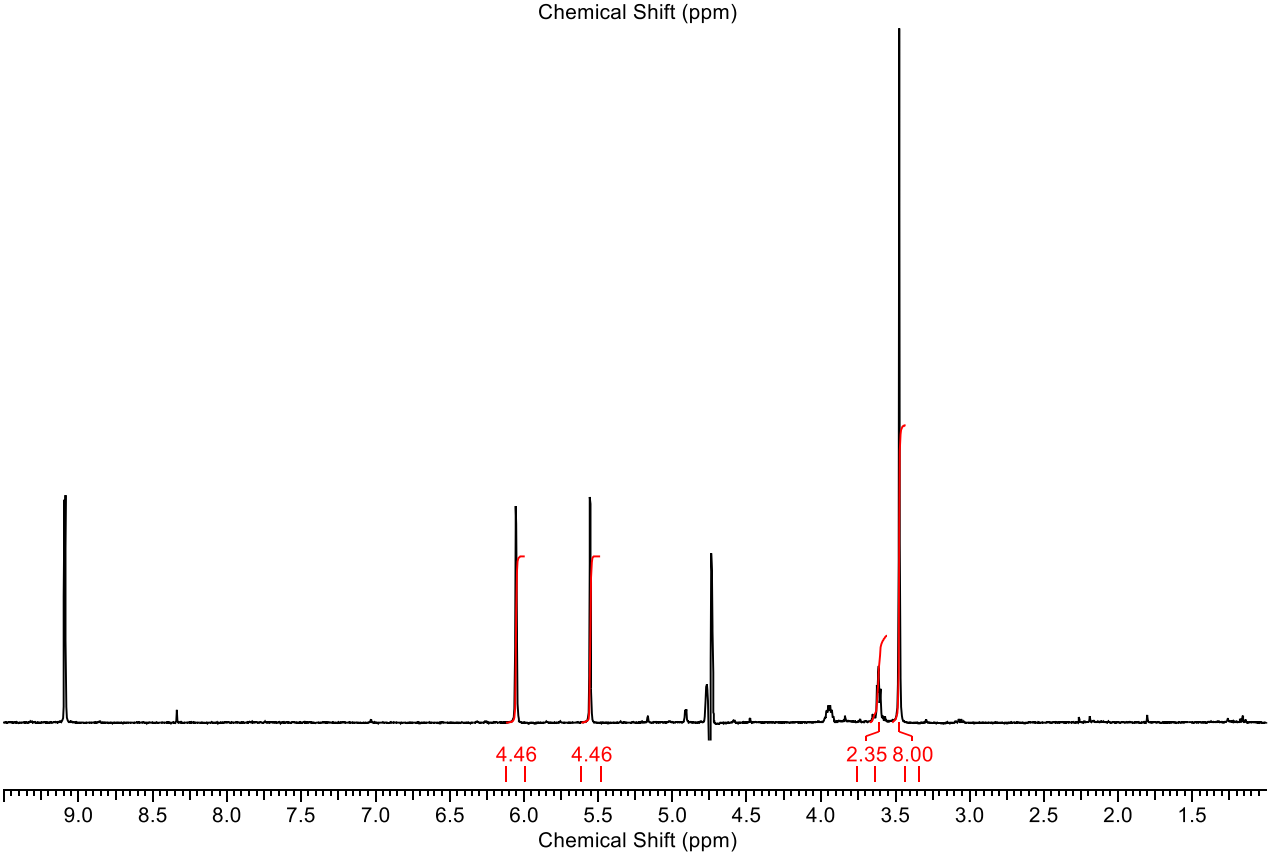
[i] Glycerate-2-phosphate solutions are neutralized (pH 7) prior to NMR acquisition. ^{1}H NMR spectra of glycerate-2-phosphate are observed to be pH dependent:
^{1}H NMR (400 MHz, {H_2O/D_2O, 9:1} pH 2) δ_H 4.61 (1H, obscured by solvent signal,CH), 3.87 (2H, br m,CH_2).
^{1}H NMR (400 MHz, {H_2O/D_2O, 9:1} pH 7) δ_H 4.39 (1H, ddd, *J* = 8.9, 5.3, 3.3 Hz, CH), 3.83 (1H, dd, *J* = 11.8, 3.3 Hz, CH_2), 3.74 (1H, *J* = 11.8, 5.3 Hz, CH_2).
^{1}H NMR (400 MHz, {H_2O/D_2O, 9:1} pH 10) δ_H 4.39 (1H, ddd, *J* = 8.6, 5.5, 2.9 Hz, CH), 3.84 (1H, dd, *J* = 11.6, 2.9 Hz, C 3.72 (1H, dd, *J* = 11.6, 5.5 Hz, CH_2).

A22: ^{1}H NMR spectra (400 MHz, {H_2O/D_2O 9:1}, 1.0–9.5 ppm) of **Top**, glyceraldehyde-2-phosphate (**29**, 65mM) with pentaerythritol internal standard and **Bottom**, after incubation at 60 ºC, pH 10, for 71.25 h.

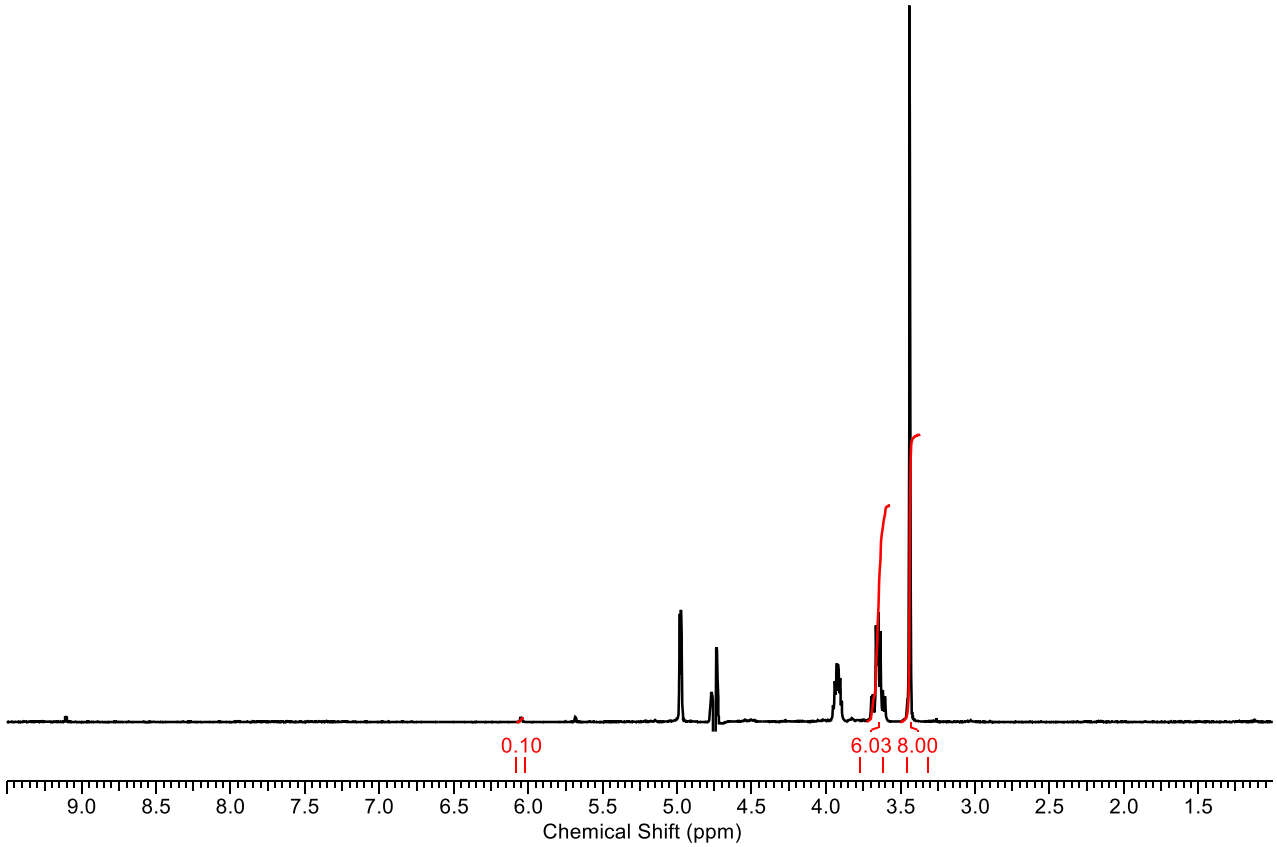


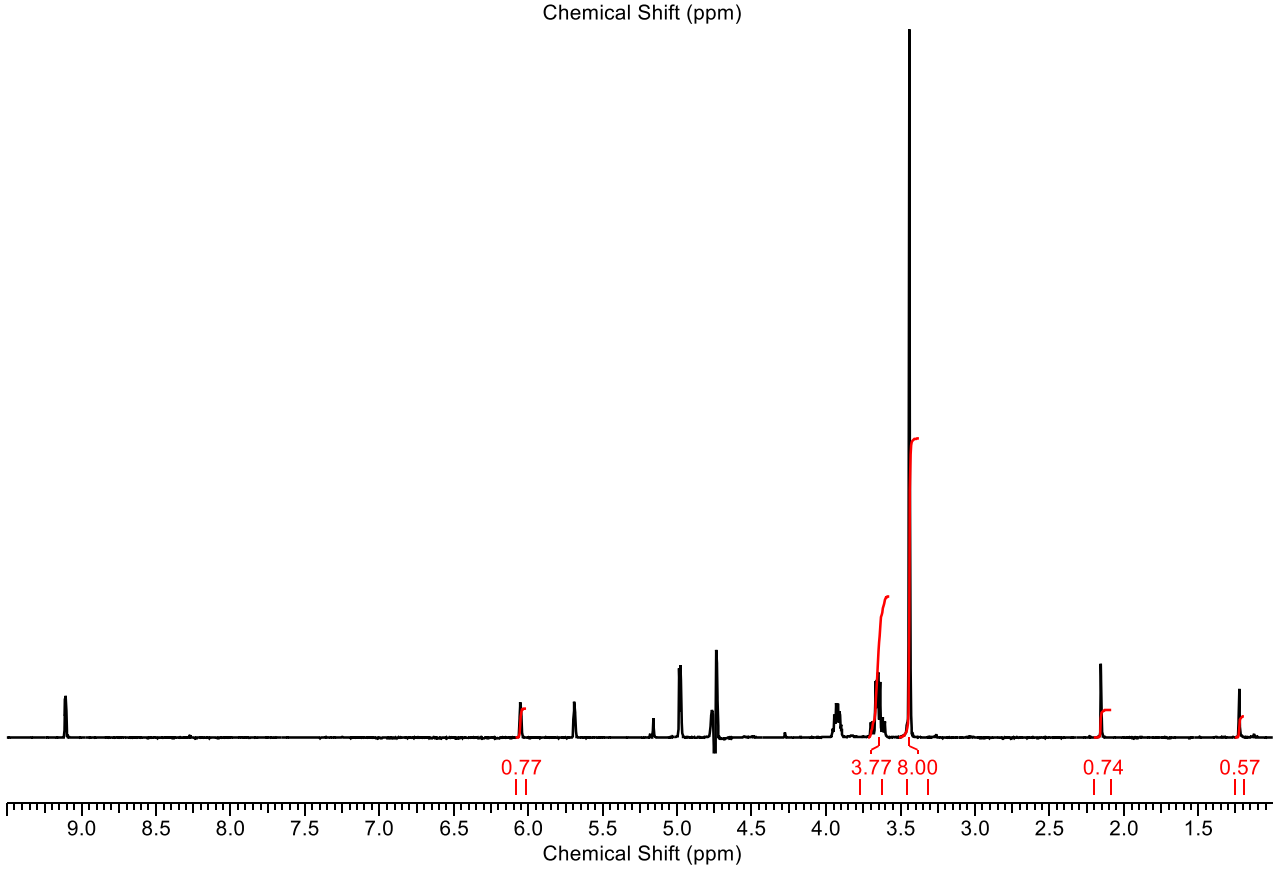
A23: ^{1}H NMR spectra (400 MHz, {500mM phosphate H_2O/D_2O 9:1}, 1.0–9.5 ppm) of **Top**, glyceraldehyde-2-phosphate (**29**, 85mM) with pentaerythritol internal standard and **Bottom**, after incubation at 60 ºC, pH 7, for 23.3 h.



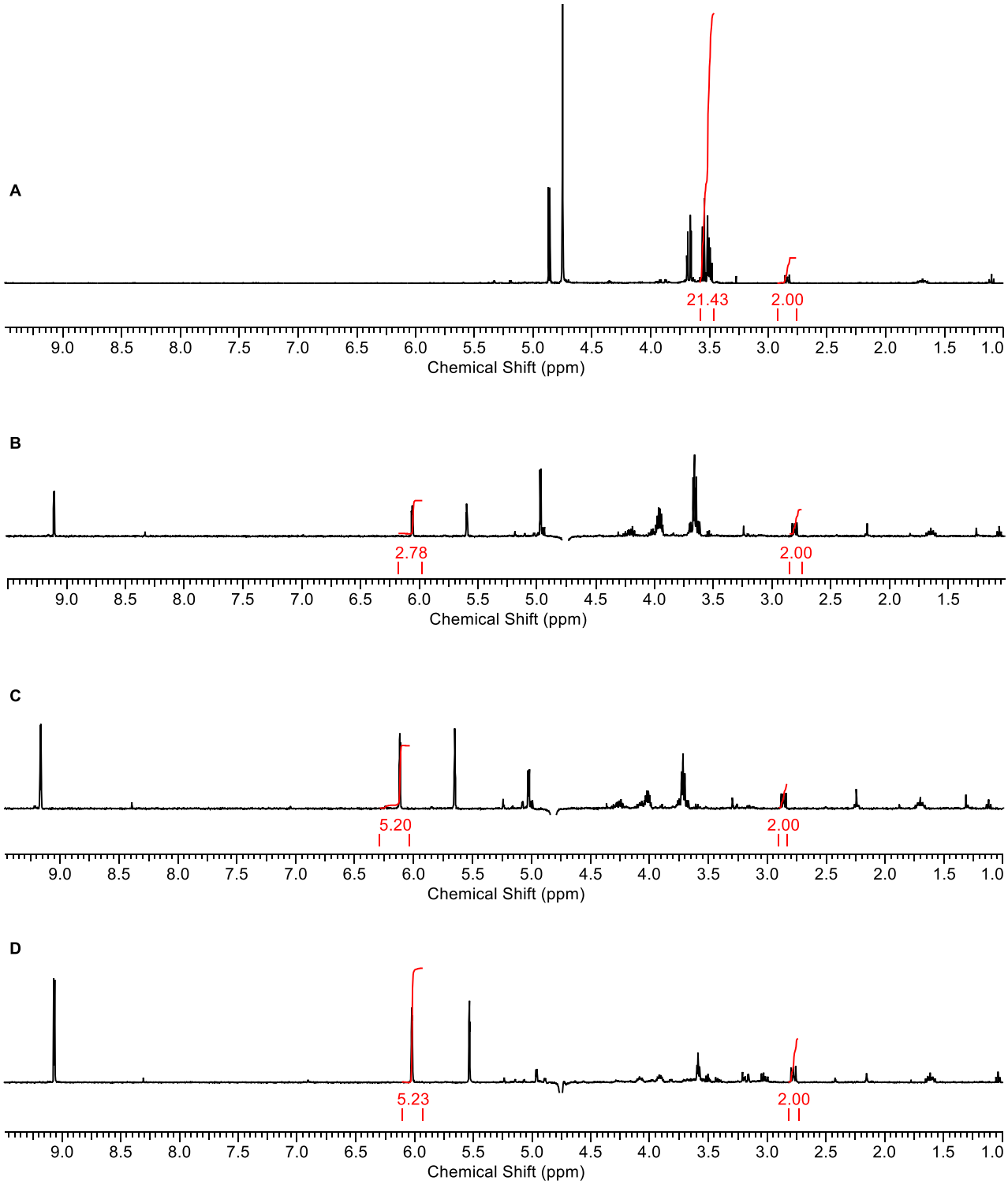


A24: ^{1}H NMR spectra (400 MHz, {1M phosphate H_2O/D_2O 9:1}, 1.0–9.5 ppm) of **Top**, glyceraldehyde-2-phosphate (**29**, 65mM) with pentaerythritol internal standard and **Bottom**, after incubation at 60 ºC, pH 4, for 17.1 h.



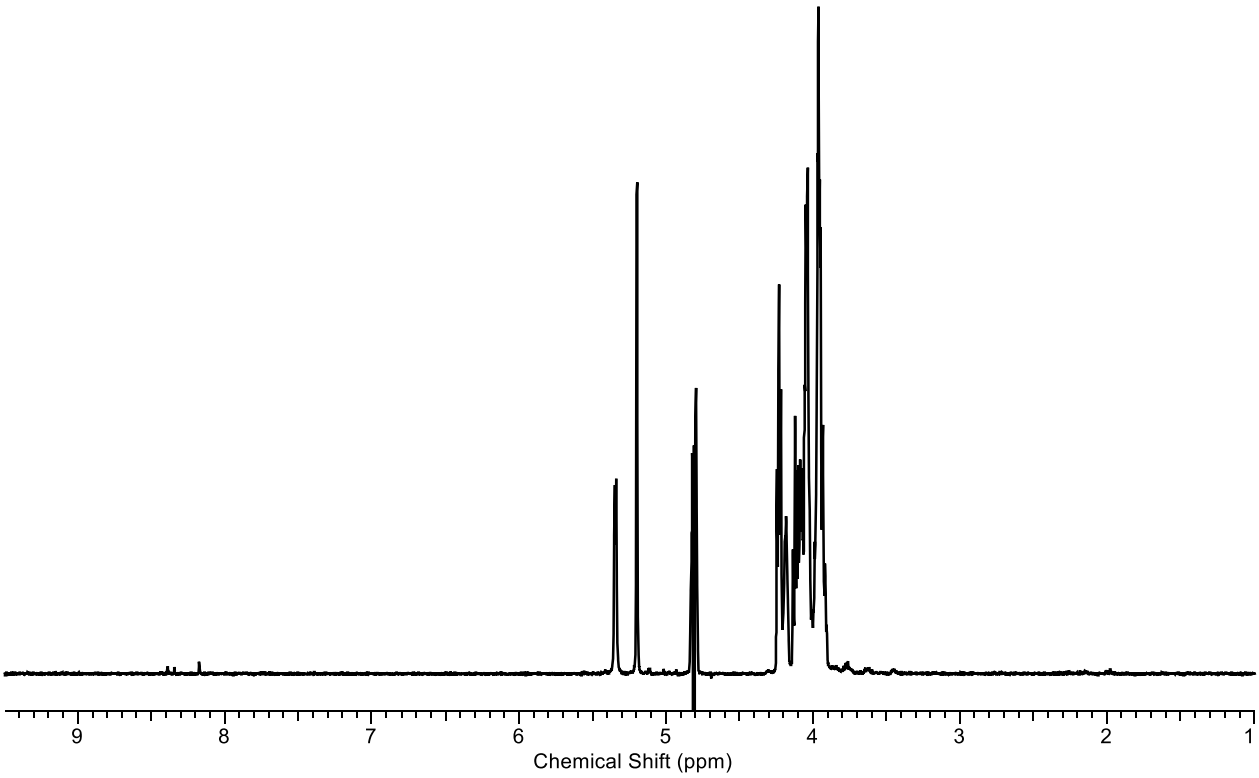


A25: ^{1}H NMR spectra (400 MHz, {750mM phosphate H_2O/D_2O 9:1}, 1.0–9.5 ppm) of **A**, glyceraldehyde (**27**, 25mM) with DSS internal standard, **B**, after incubation with diamidophosphate **48** (100mM) at RT, pH 4, for 22 days, **C**, after an additional 3 h at 60 °C and **D**, an aliquot of the original sample after reaction with diamidophosphate (**48**) at pH 4, RT for 4 h followed by readjustment to pH 7 and incubation at 60 °C for 4.5 h.



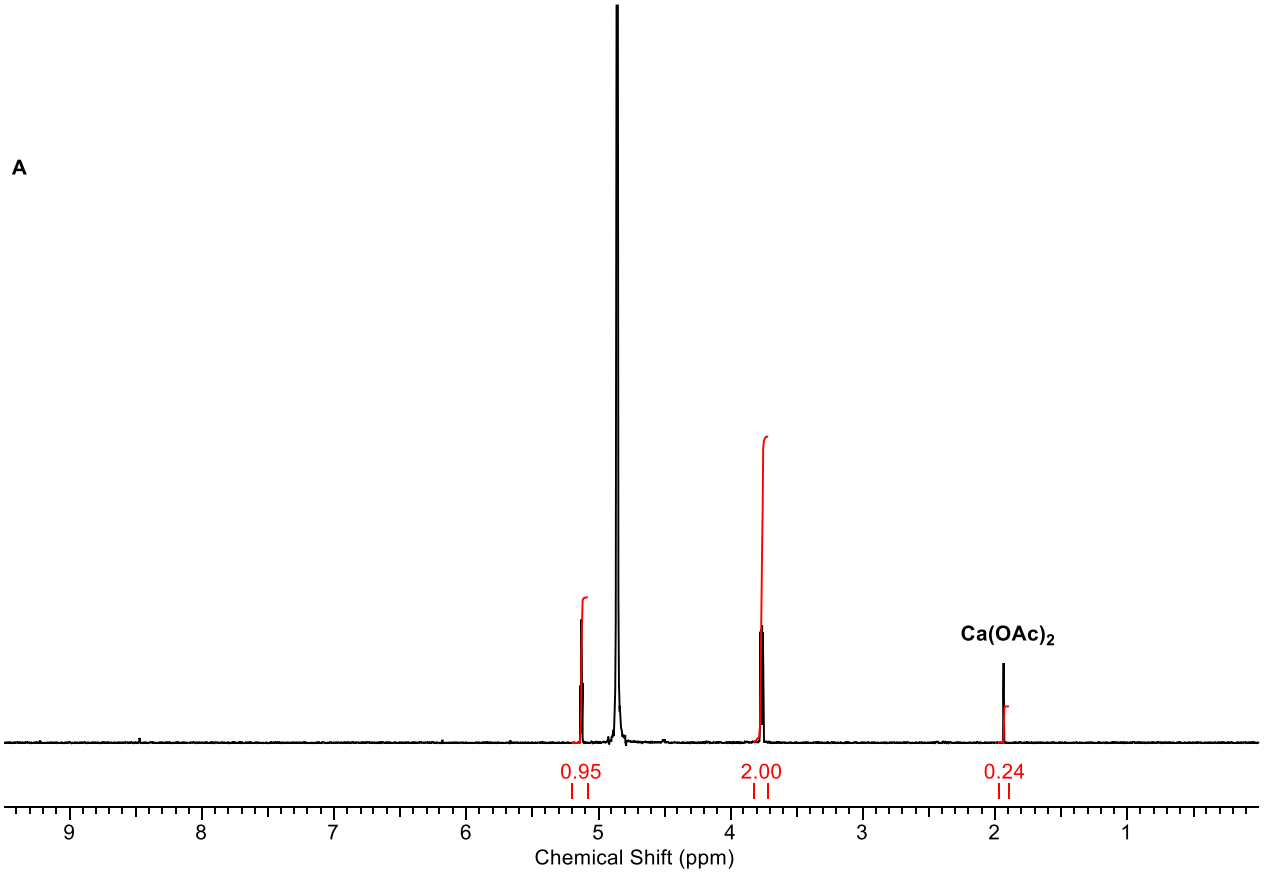
Ribose-5′-phosphate (**107**)

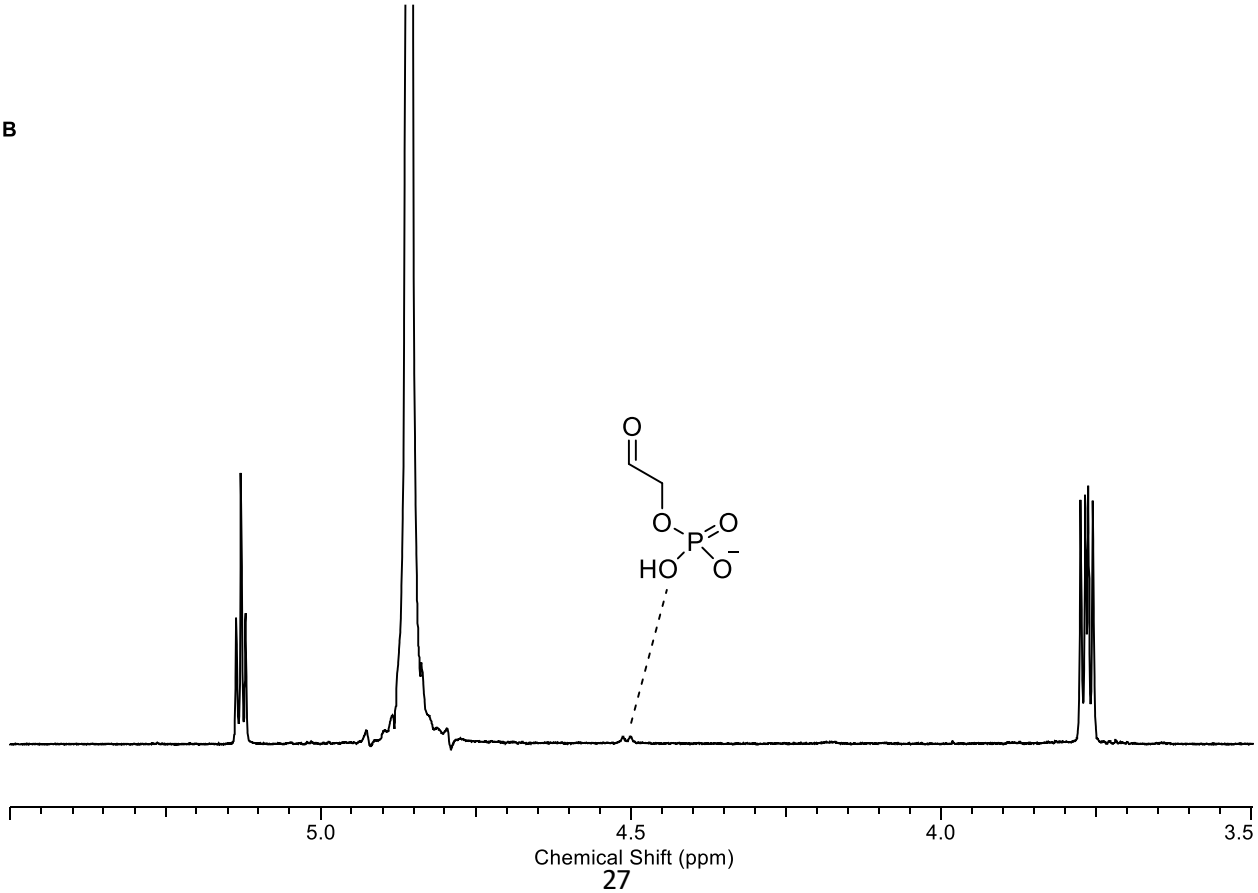
A26: ^{1}H NMR spectrum (400 MHz, {H_2O/D_2O 9:1}, 1.0 – 9.5 ppm) of ribose-5′-phosphate (**107**) formed from the Dowex™ hydrolysis of adenosine-5′-monophosphate (**14**).



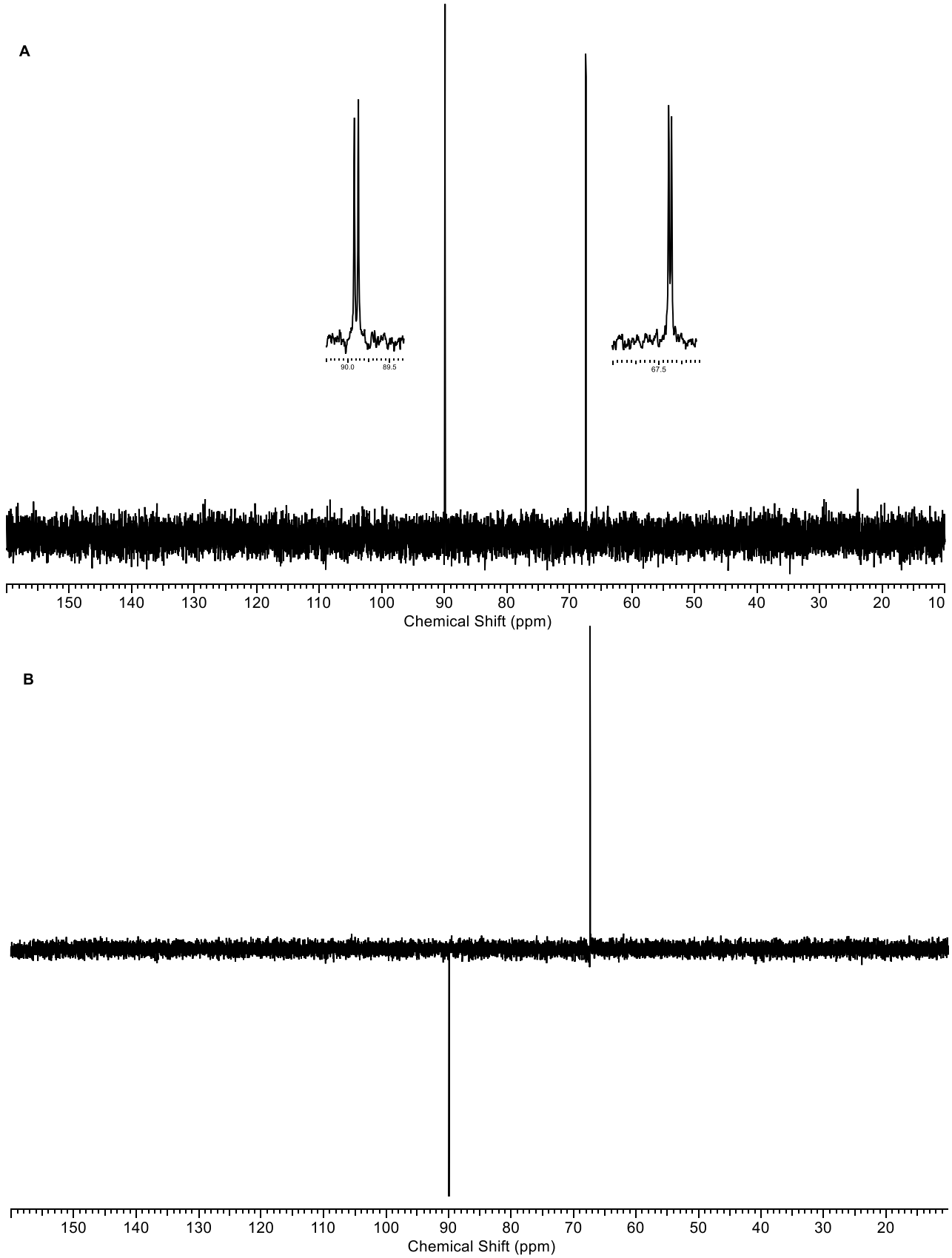
Glycolaldehyde phosphate (**28**)

A27: ^{1}H NMR spectra (600 MHz, {D_2O} **A**, 0.0–9.5 ppm and **B**, 3.5 – 5.5 ppm) of glycolaldehyde phosphate (**28**) calcium salt.

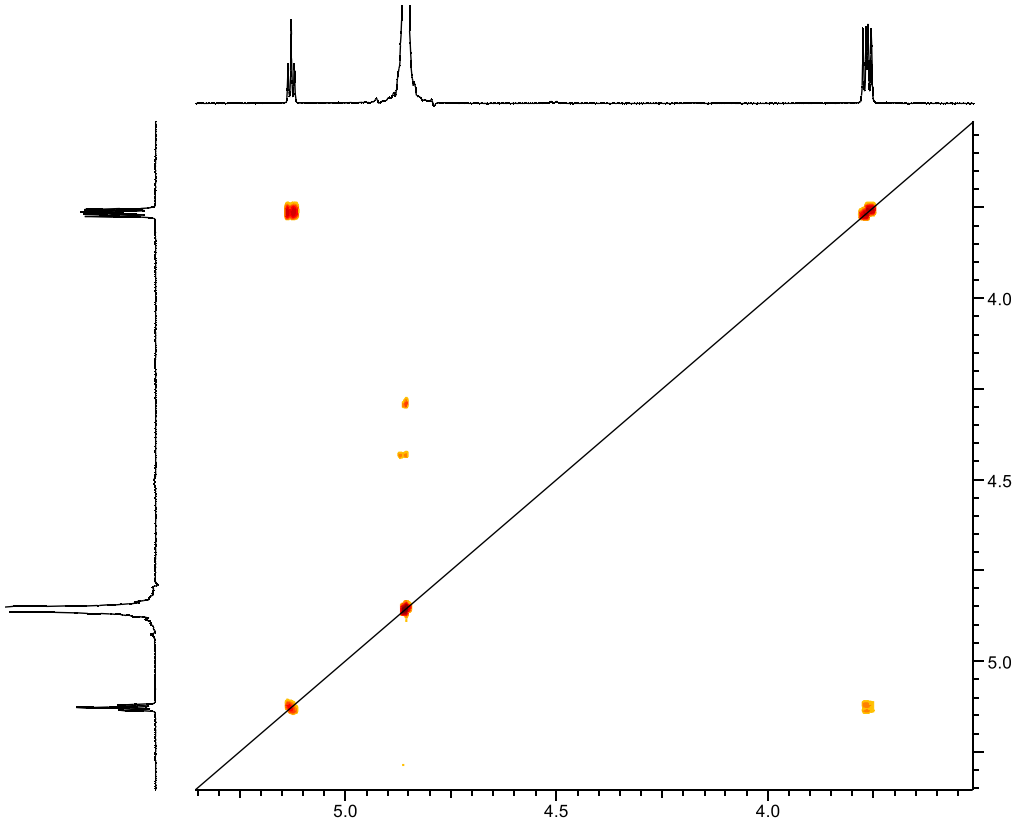




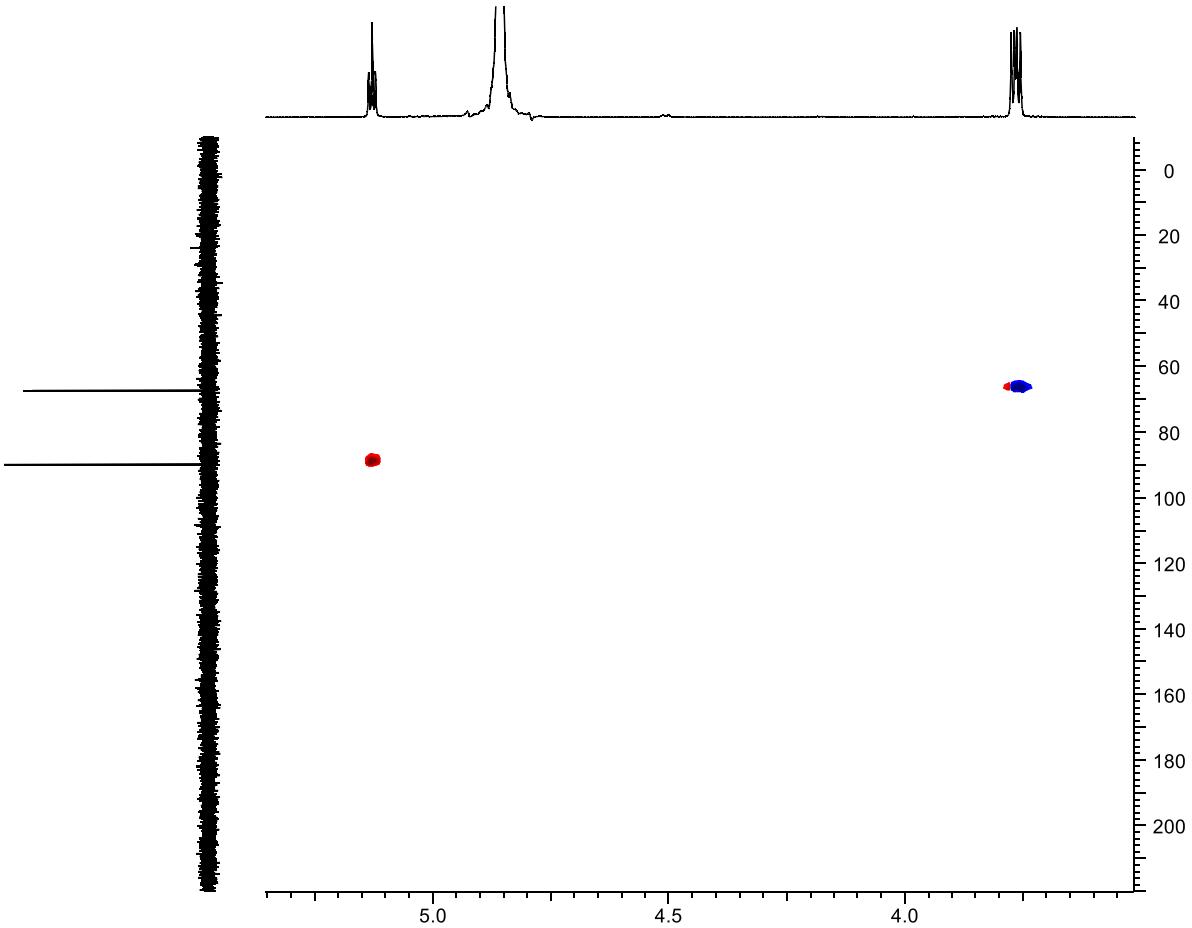
A28: **A**, ^{13}C and **B**, DEPT-135 NMR spectra (151 MHz, {D_2O} 10 – 160 ppm) of **28** calcium salt.



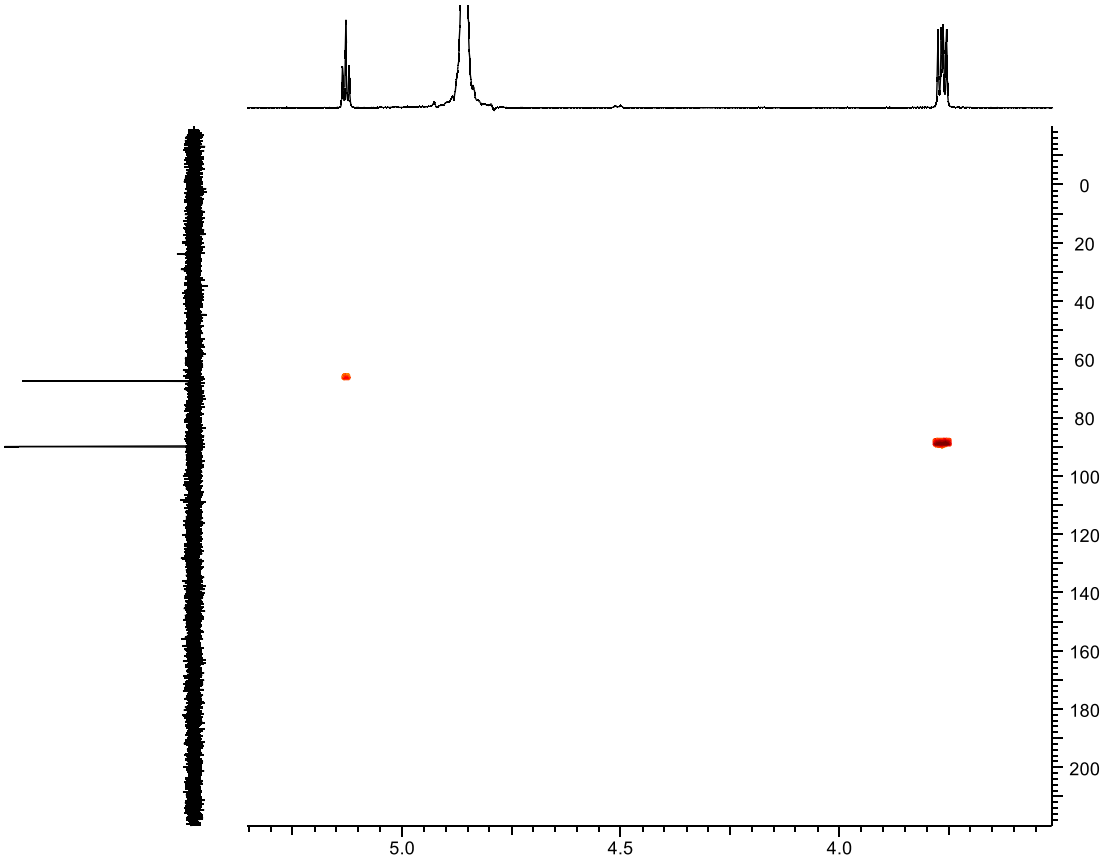
A29: ^{1}H-^{1}H COSY NMR spectrum (600 MHz, {D_2O} 3.5 – 5.5 ppm), of **28** calcium salt.



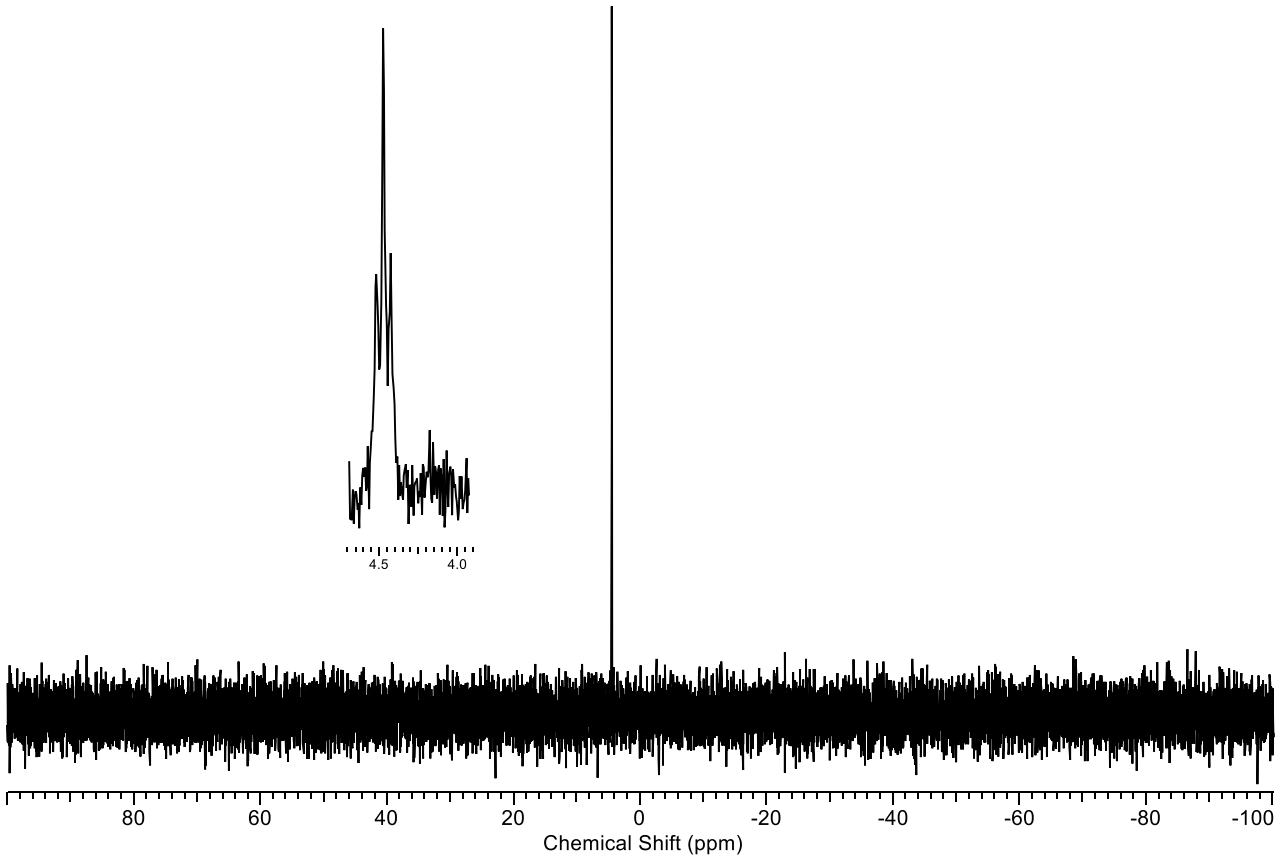
A30: ^{1}H-^{13}C HSQC NMR spectrum (600 MHz, {D_2O}) of **28** calcium salt.



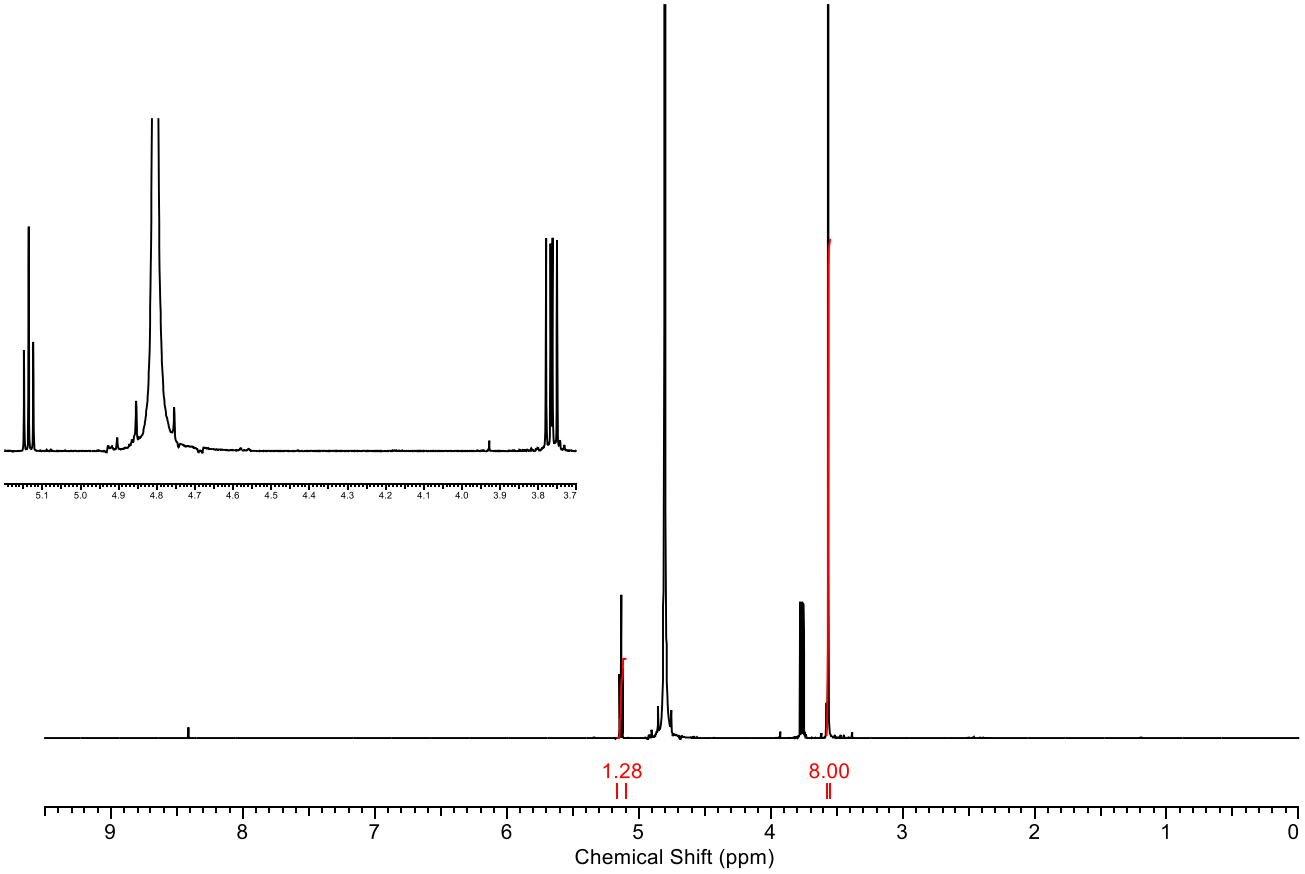
A31: ^{1}H-^{13}C HMBC NMR spectrum (600 MHz, {D_2O}) of **28** calcium salt.



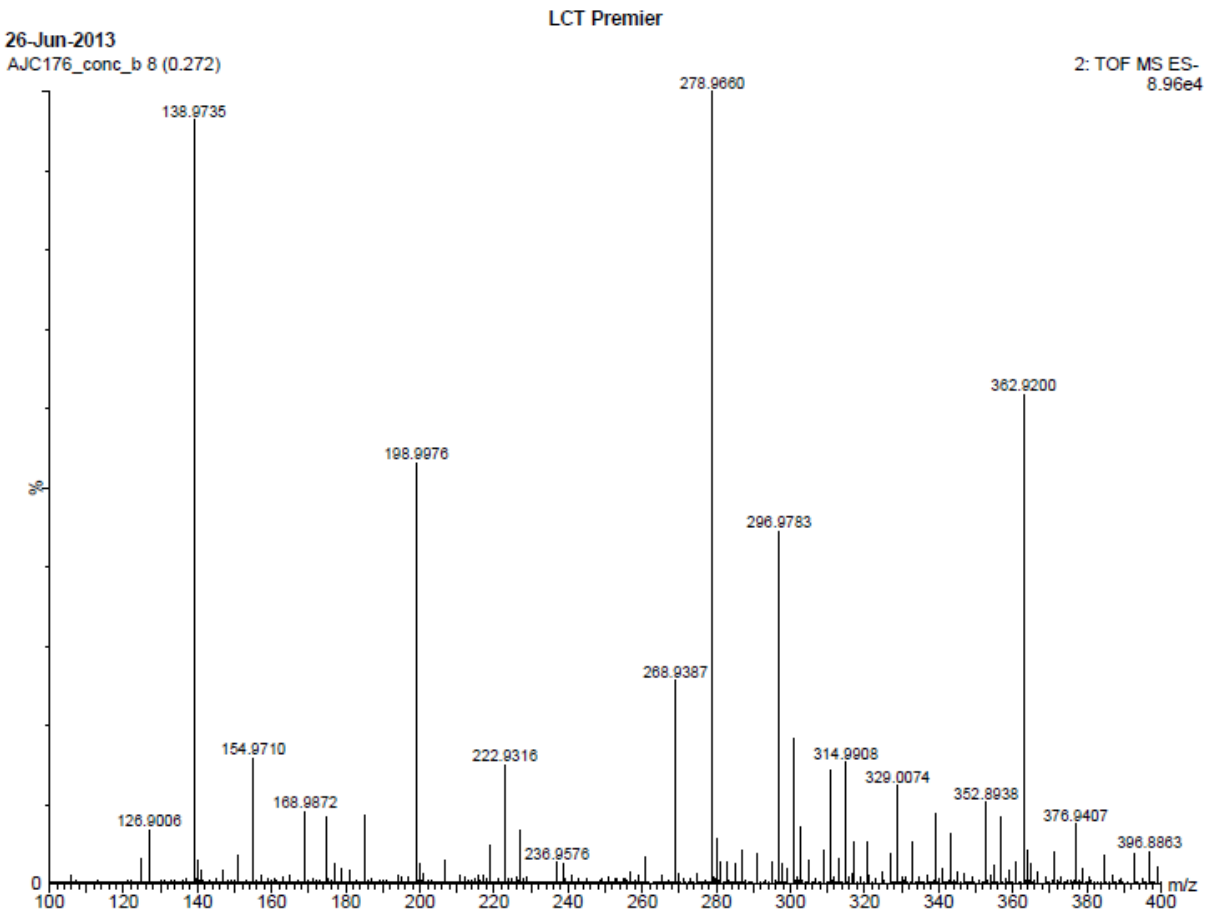
A32: ^{31}P NMR spectrum (161 MHz, {D_2O} -100 – 100 ppm) of **28** calcium salt with $^{31}P\{^{1}H$-coupled} signal overlaid.



A33: ^{1}H NMR spectra (600 MHz, {D_2O}, 0.0 – 9.5 ppm) of **28** (132mM) sodium salt with pentaerythritol, with expansion (3.7–5.2 ppm) overlaid.

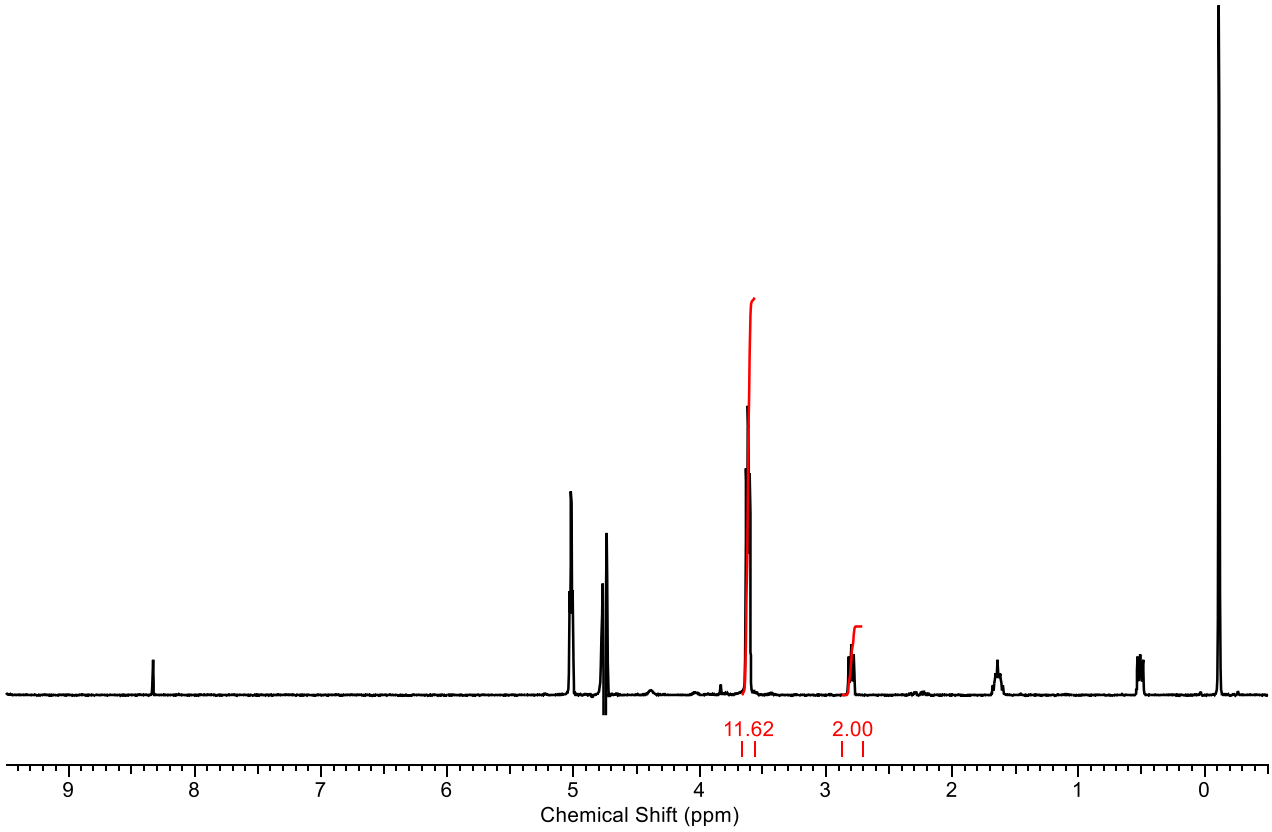


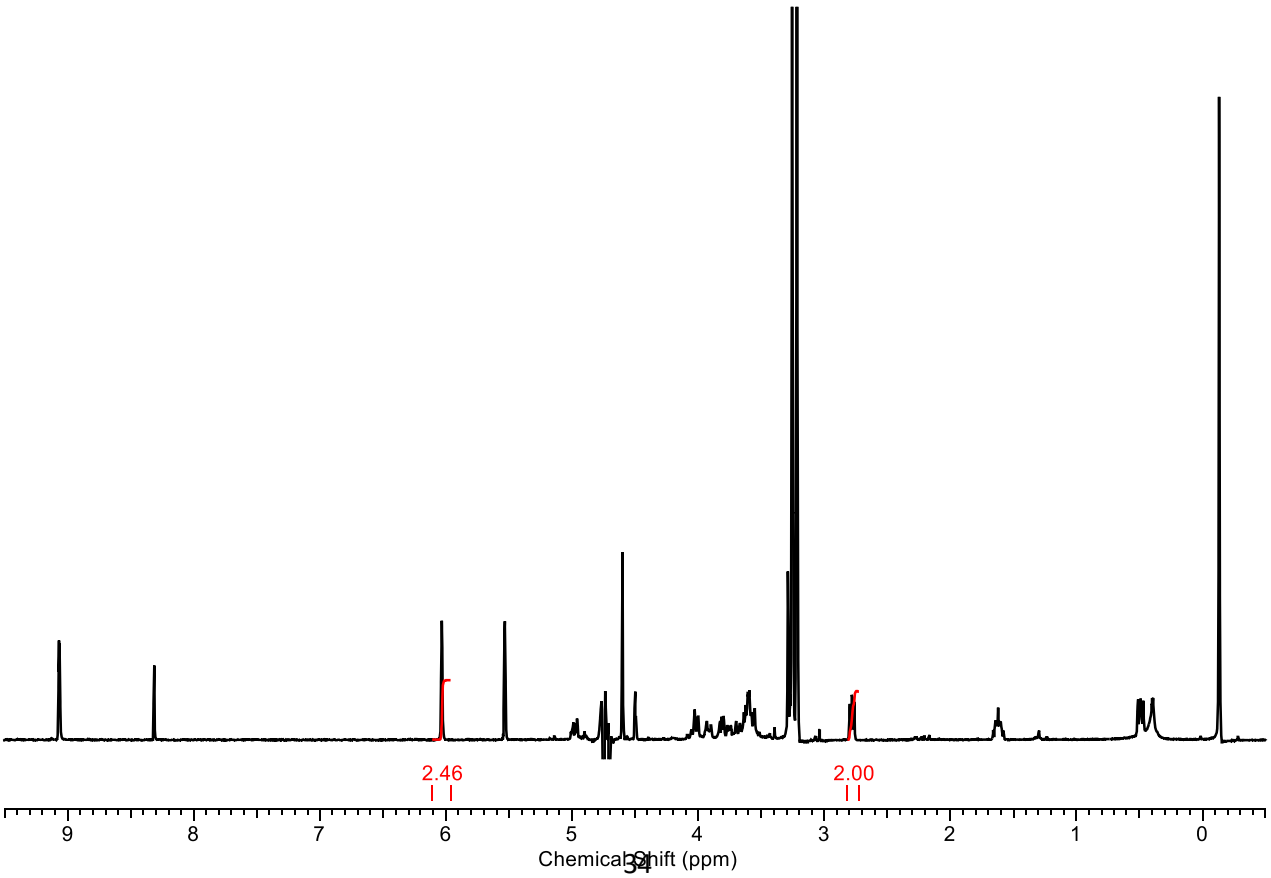
A34: ESI- mass spectrum of **28** sodium salt solution.



Reaction of glycolaldehyde phosphate (**28**) with formaldehyde

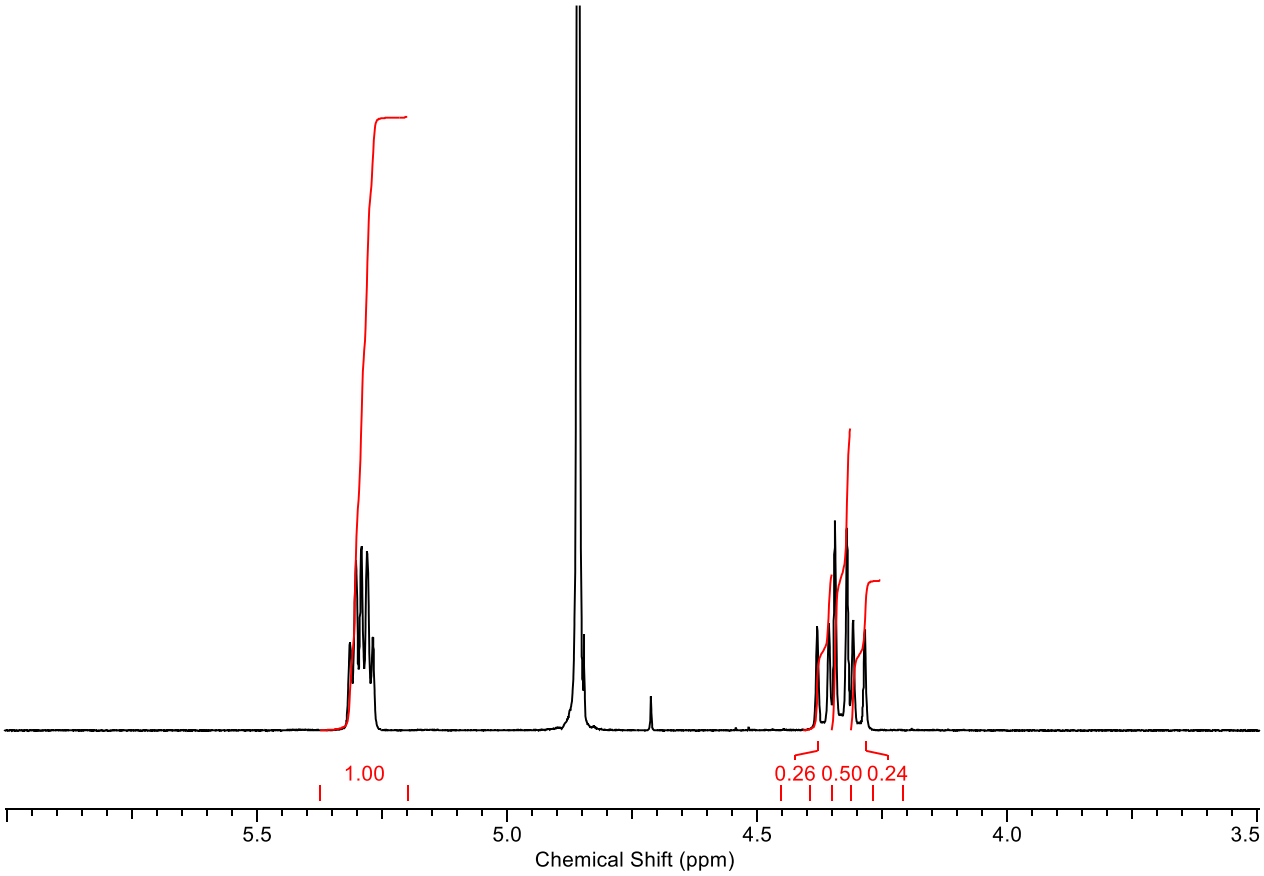
A35: ^{1}H NMR spectra (400 MHz, {500mM phosphate H_2O/D_2O, 9:1}, -0.5 – 9.5 ppm) of **Top**, glycolaldehyde-2-phosphate (**28**, 80mM) with DSS and **Bottom**, after incubation with formaldehyde (500mM) at 60 °C, pH 7, 29.8 h.

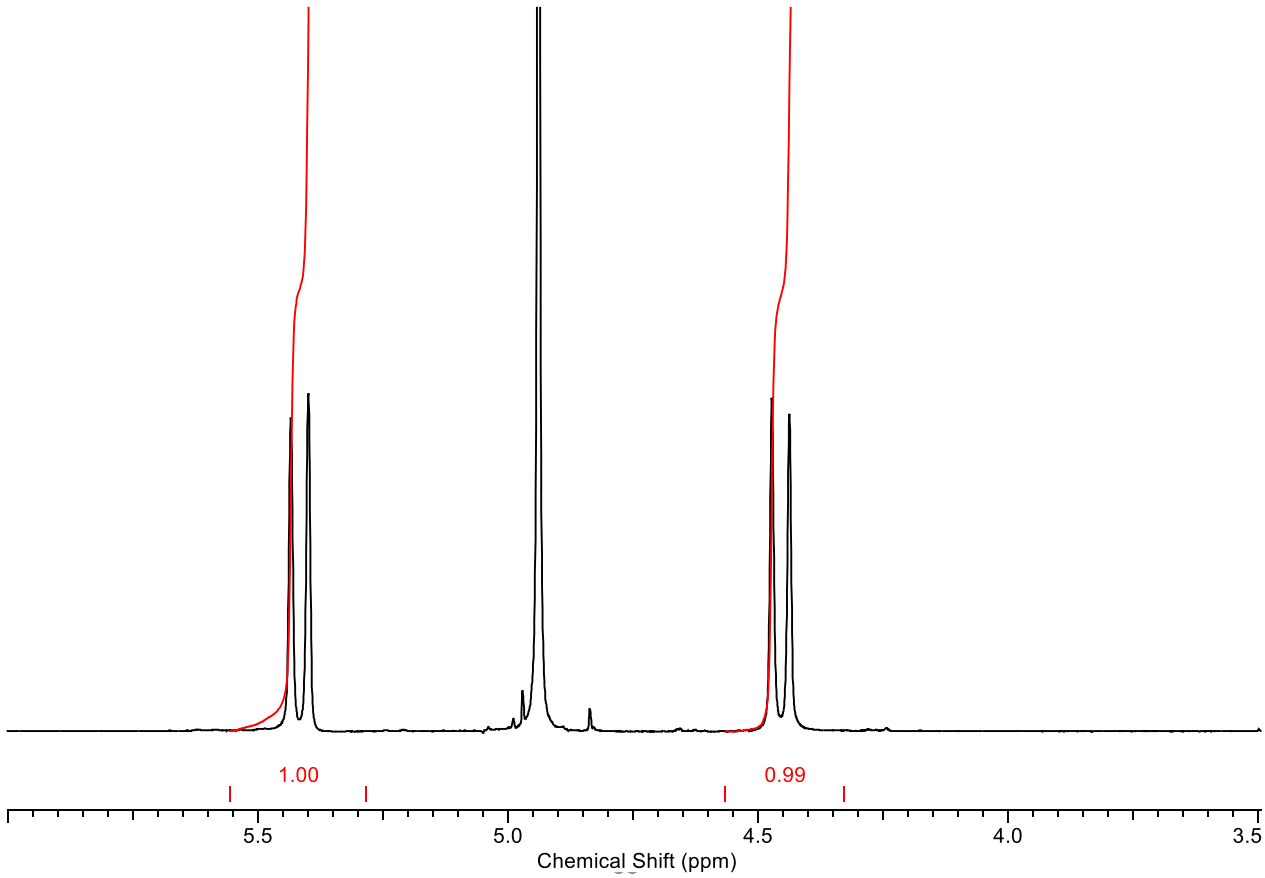




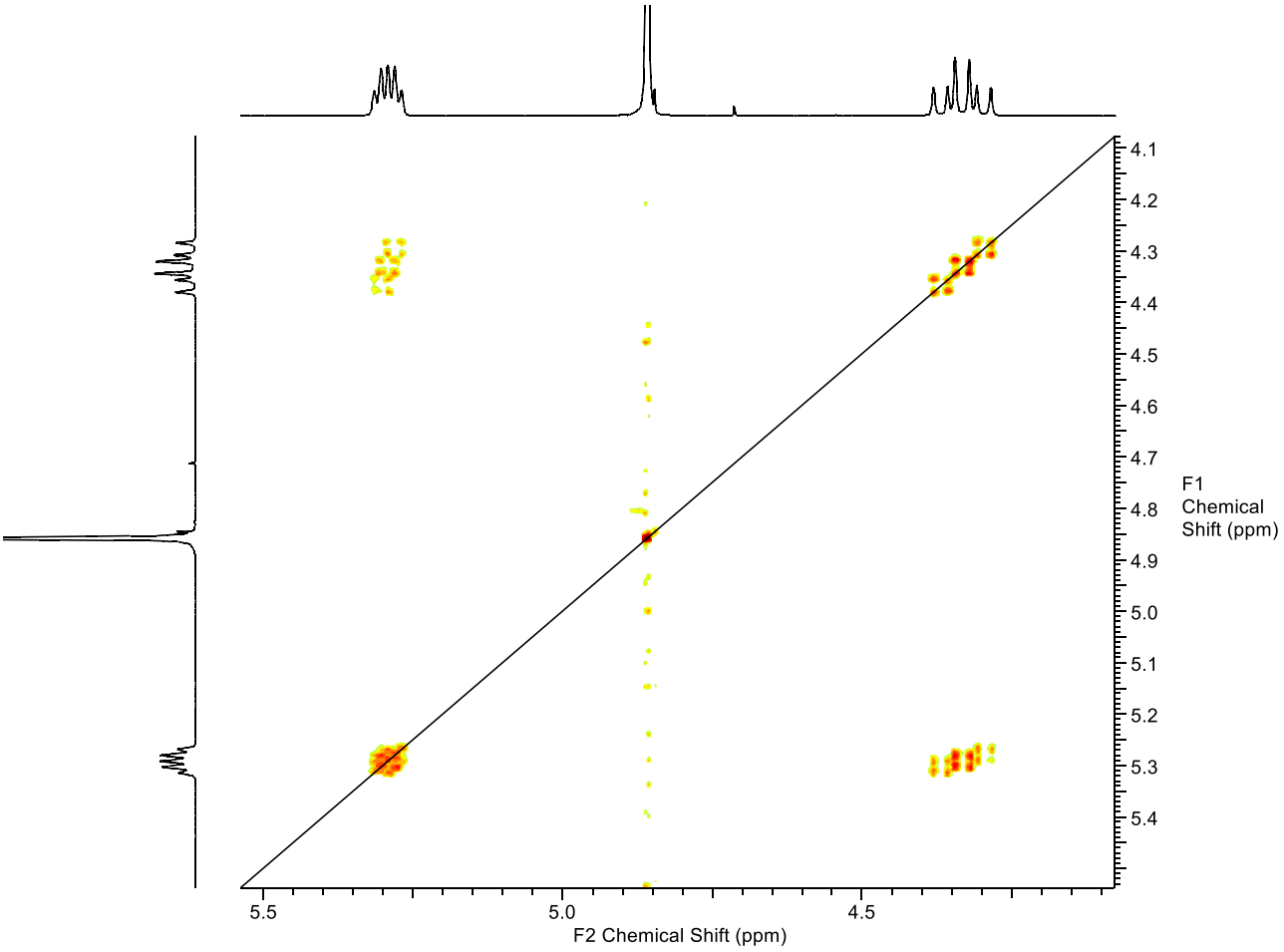
Diamidophoshate-formaldhehyde adduct (**108**)

A36: **Top**, ^{1}H NMR spectrum (400 MHz, {D2O}, 3.5 – 6.0 ppm) and **Bottom**, ^{1}H NMR {^{31}P-decoupled} spectrum (400 MHz, D2O, 3.5 – 6.0 ppm) of **108** sodium salt.

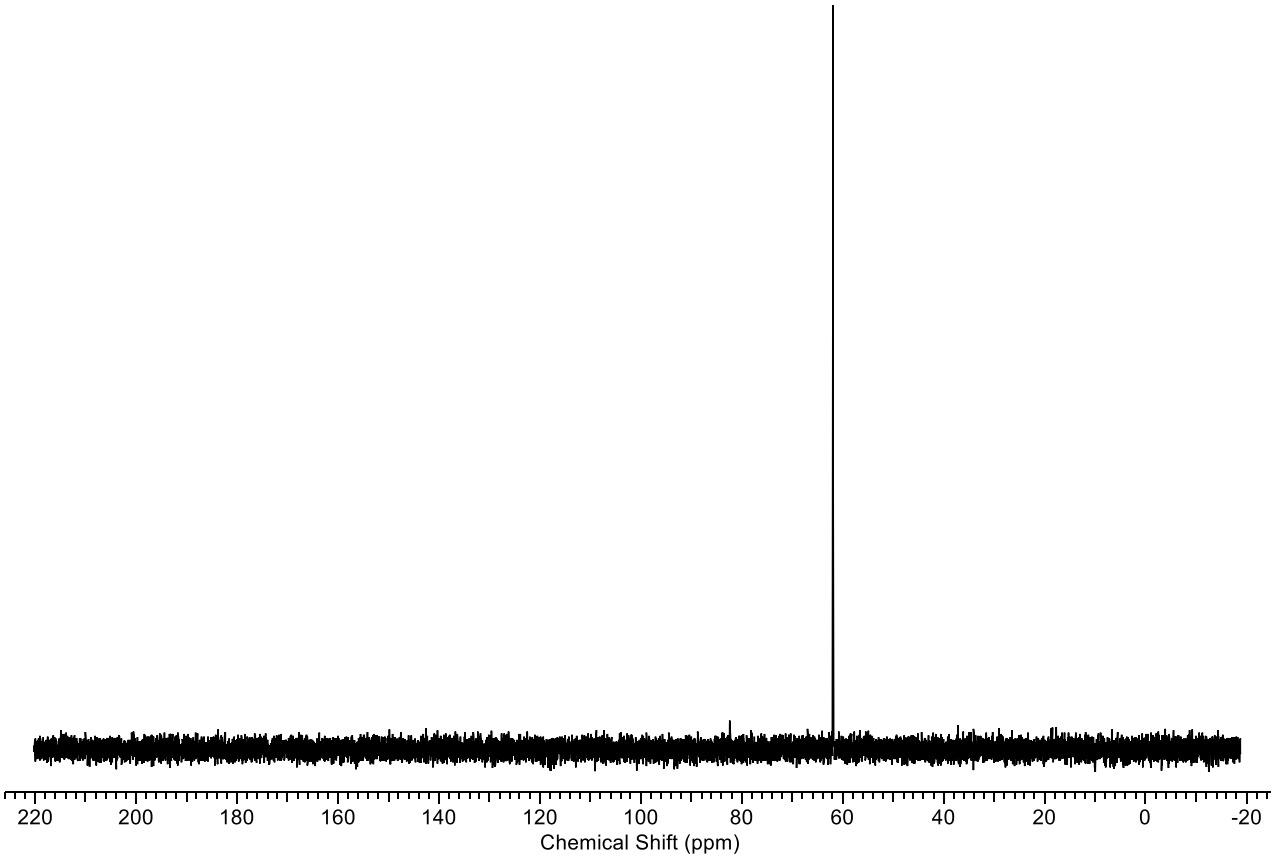




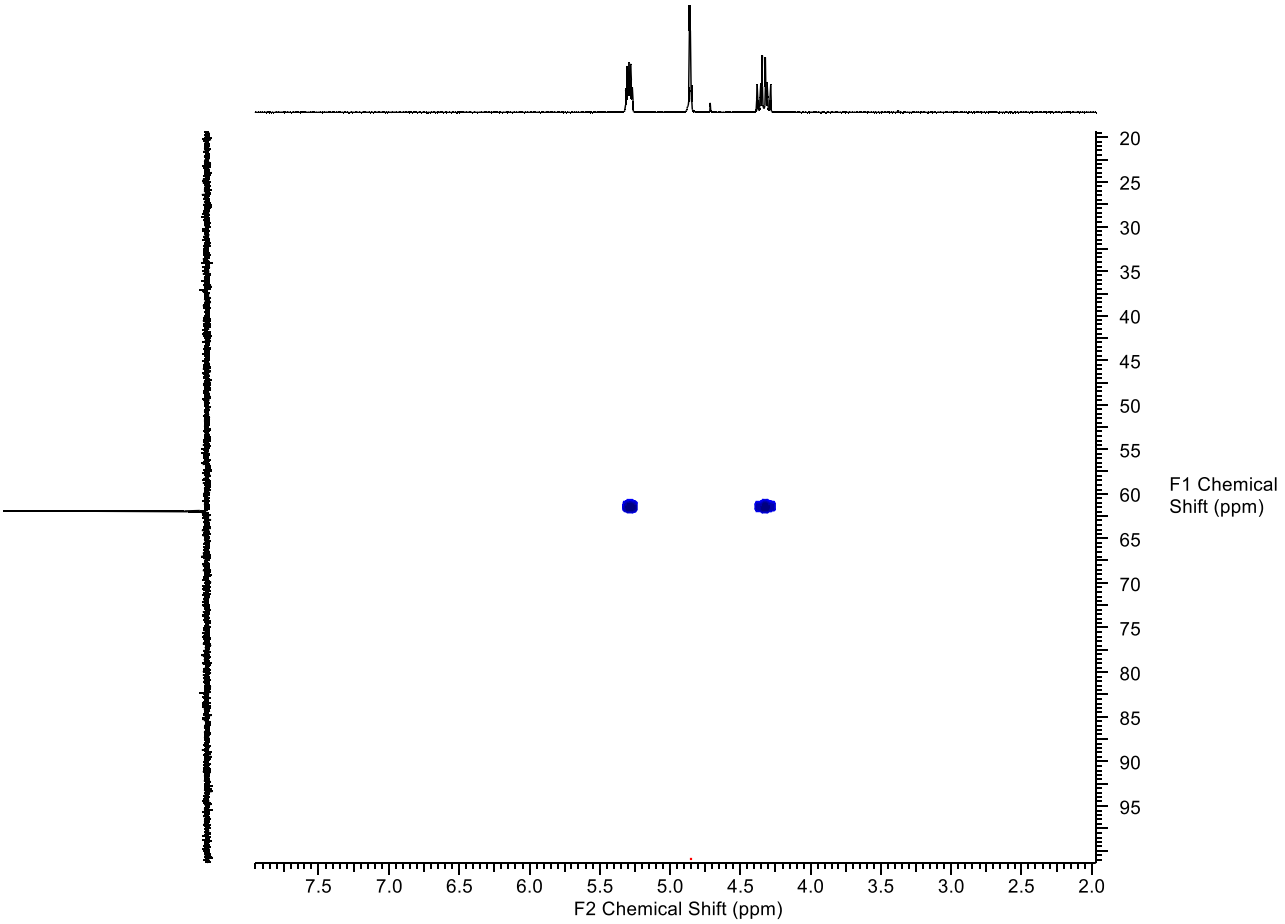
A37: ^{1}H-^{1}H COSY NMR spectrum (600 MHz, {D2O} 4.0 – 5.5 ppm), of **108** sodium salt



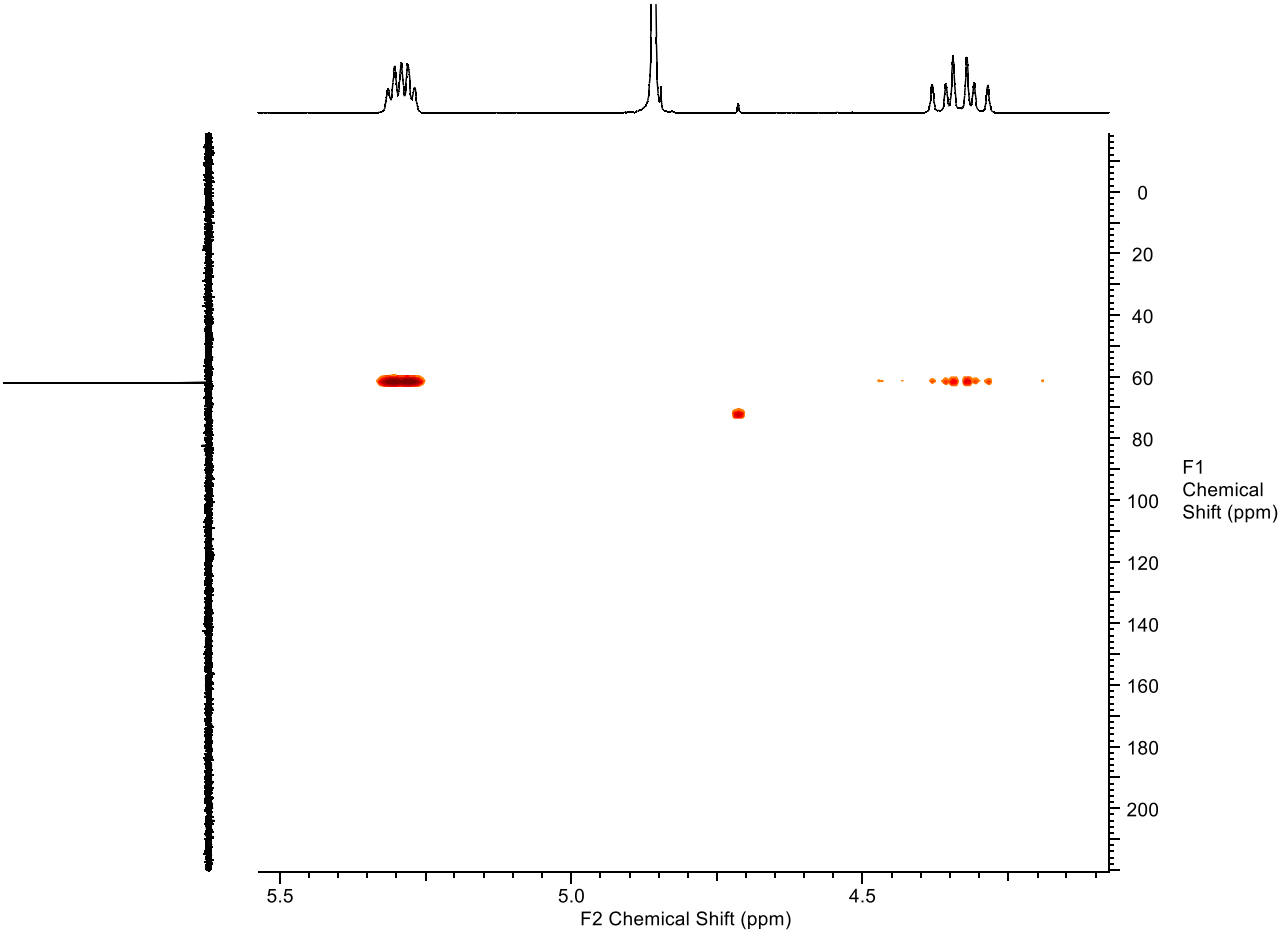
A38: ^{13}C NMR spectrum (151 MHz, {D2O} -20 – 220 ppm) of **108** sodium salt.



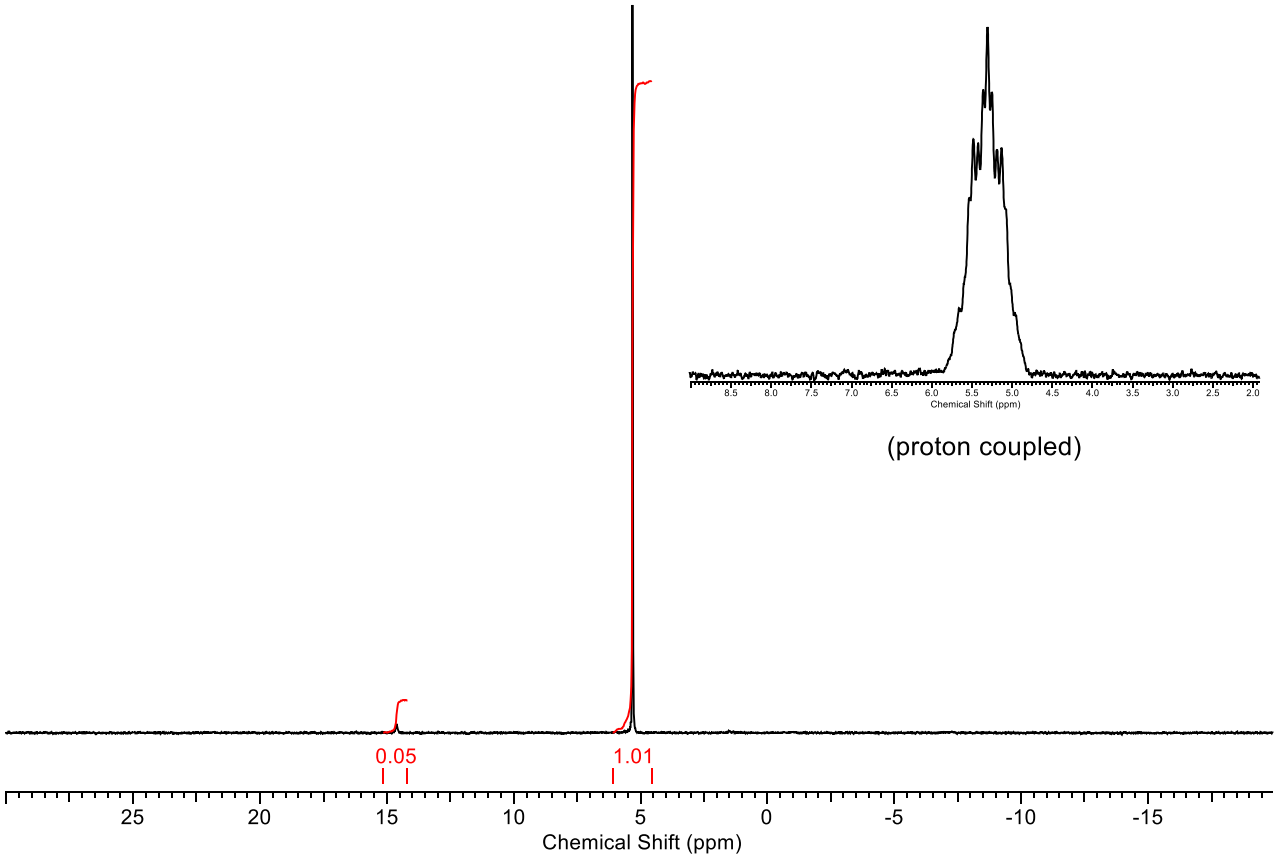
A39: ^{1}H-^{13}C HSQC NMR spectrum (600 MHz, {D_2O}) of **108** sodium salt.



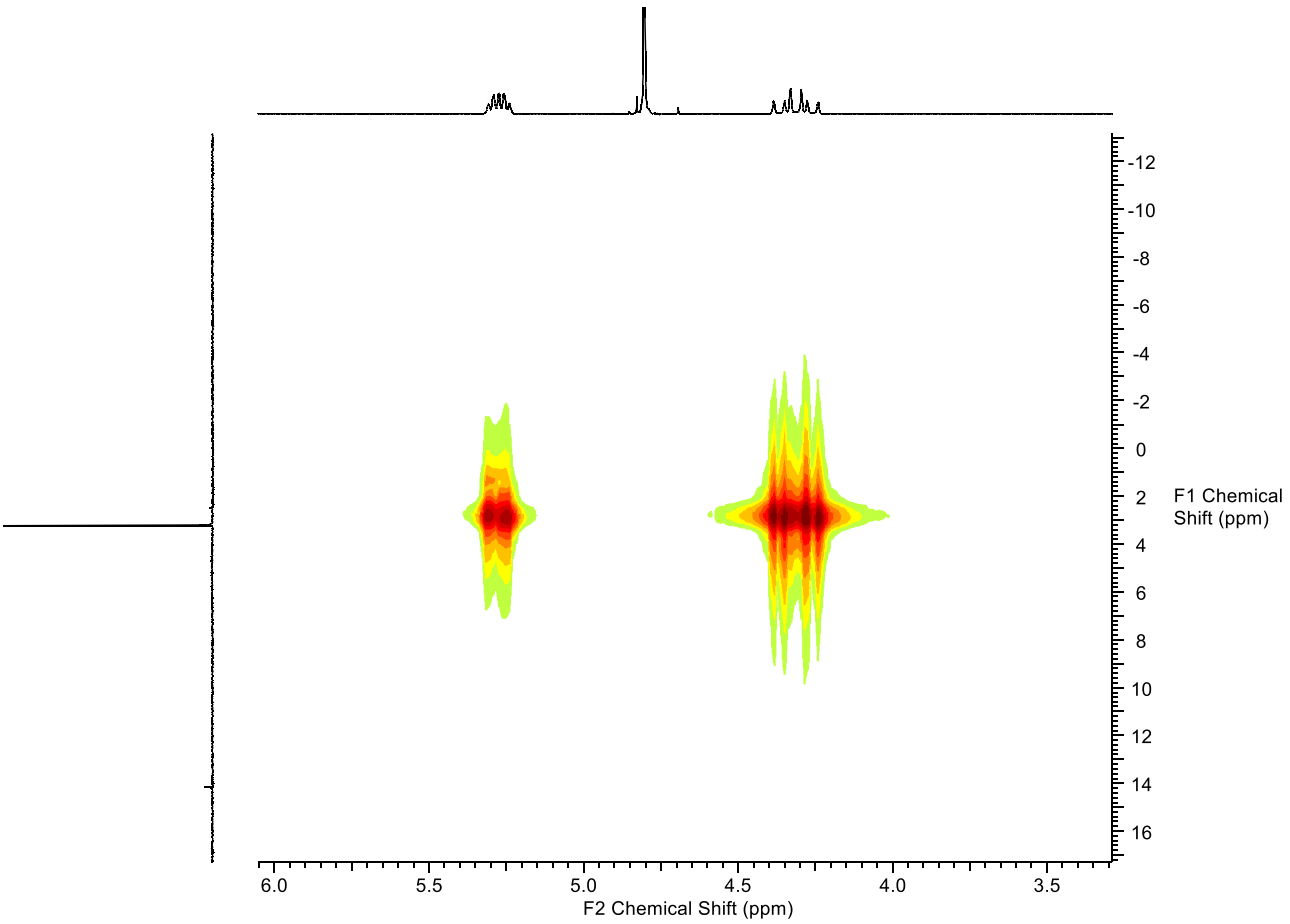
A40: ^{1}H-^{13}C HMBC NMR spectrum (600 MHz, {D_2O}) of **108** sodium salt.



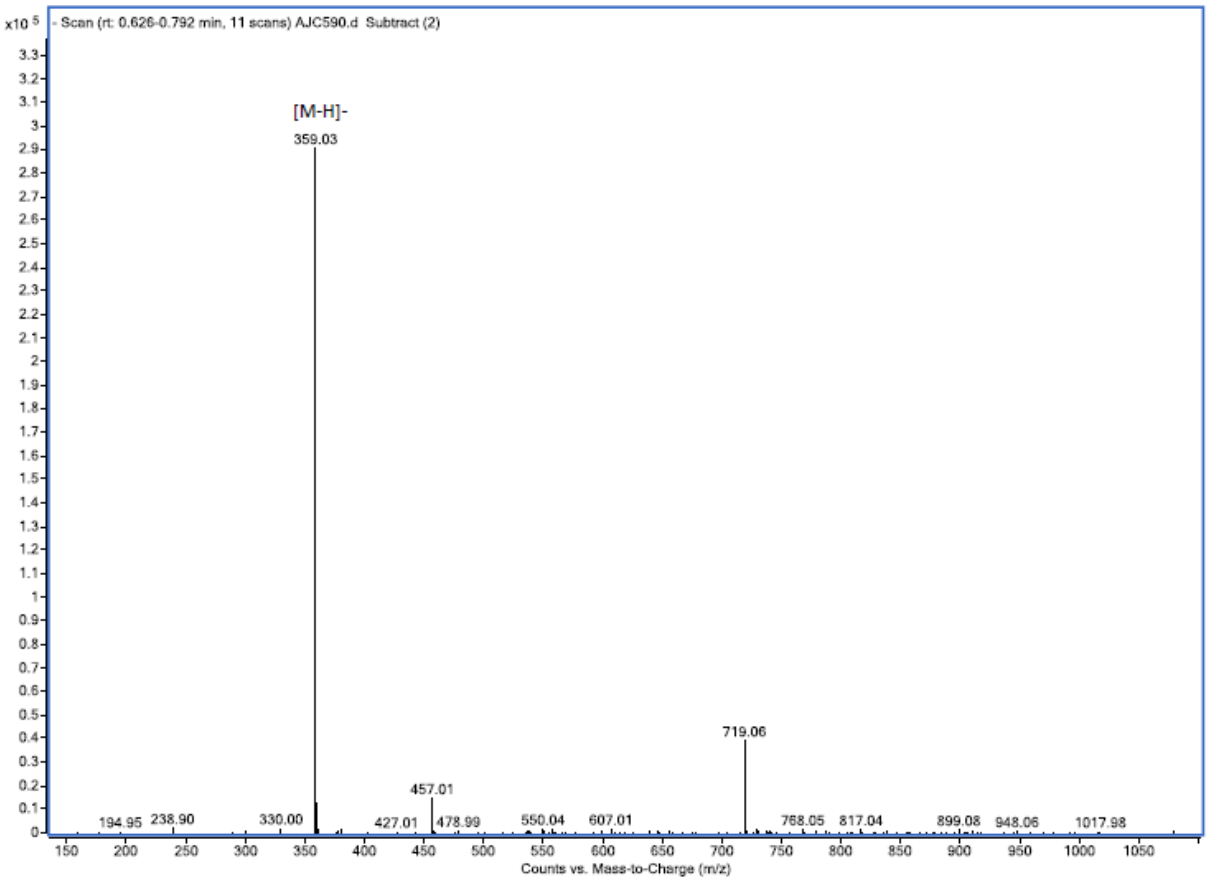
A41: ^{31}P NMR spectrum (161 MHz, {D_2O} -20 – 30 ppm) of **108** sodium salt with $^{31}P\{^{1}H$-coupled} signal overlaid.



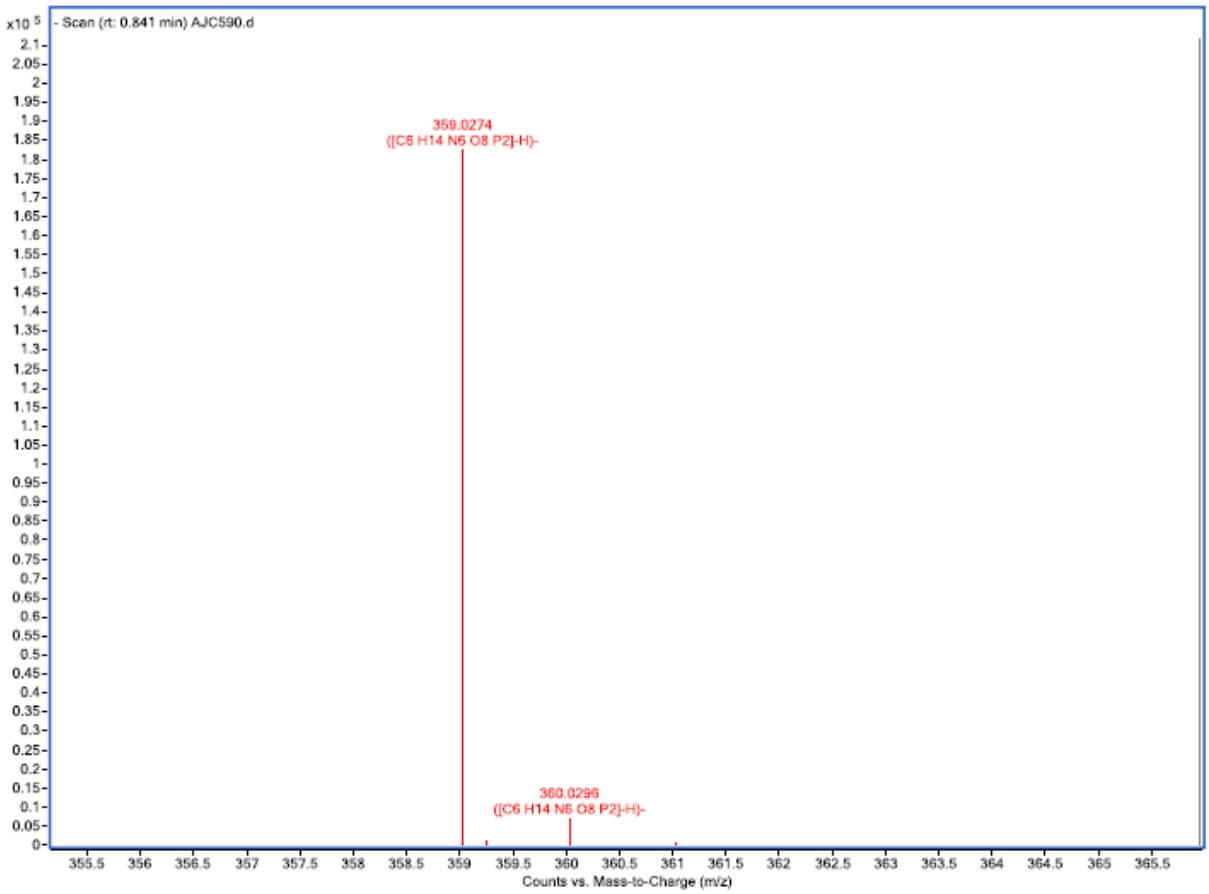
A42: ^{1}H-^{31}P HMBC NMR spectrum (400 MHz, {D_2O}) of **108** sodium salt.



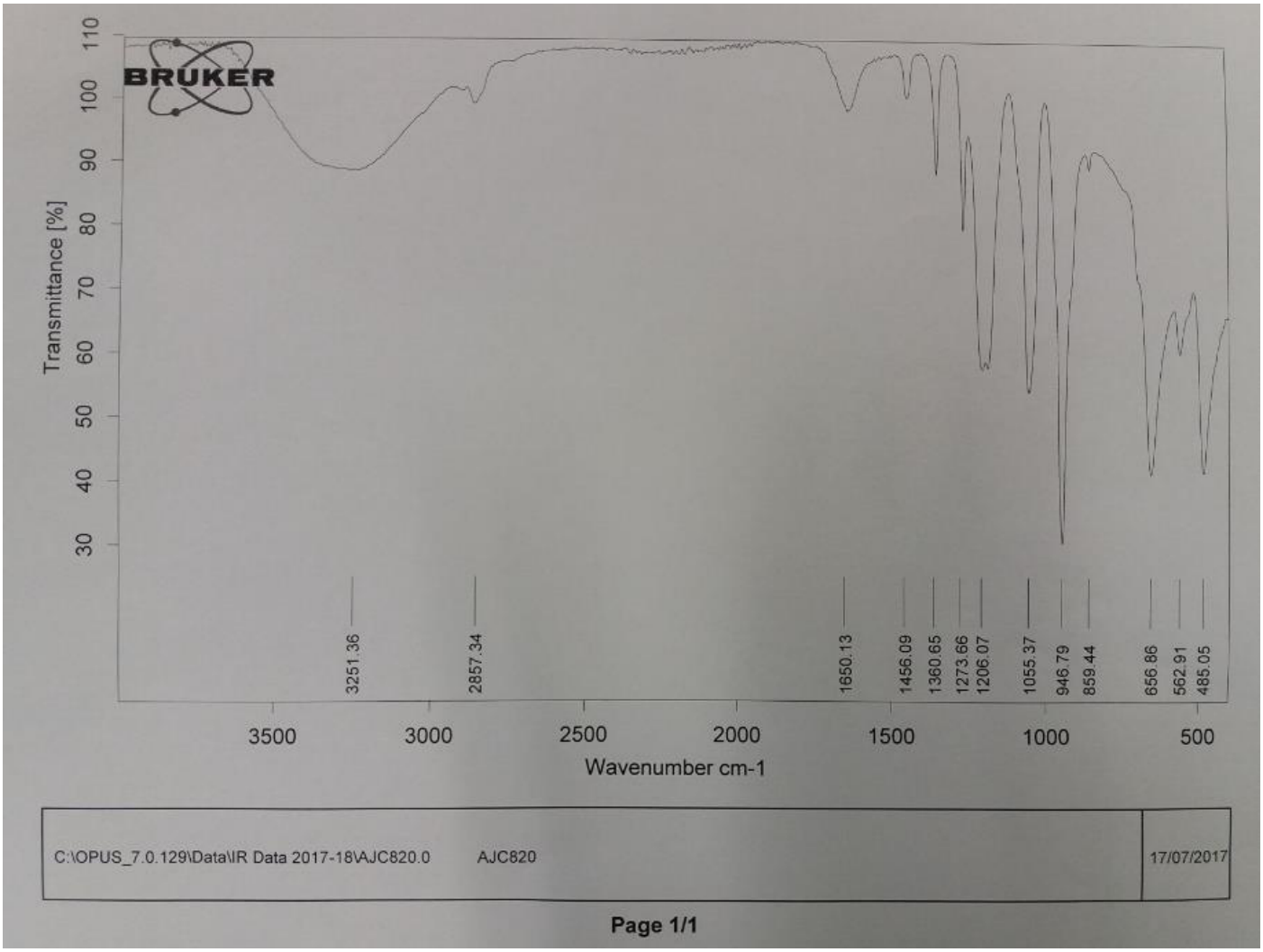
A43: ESI- mass spectrum of **108** sodium salt.



A44: HRMS of **108**.

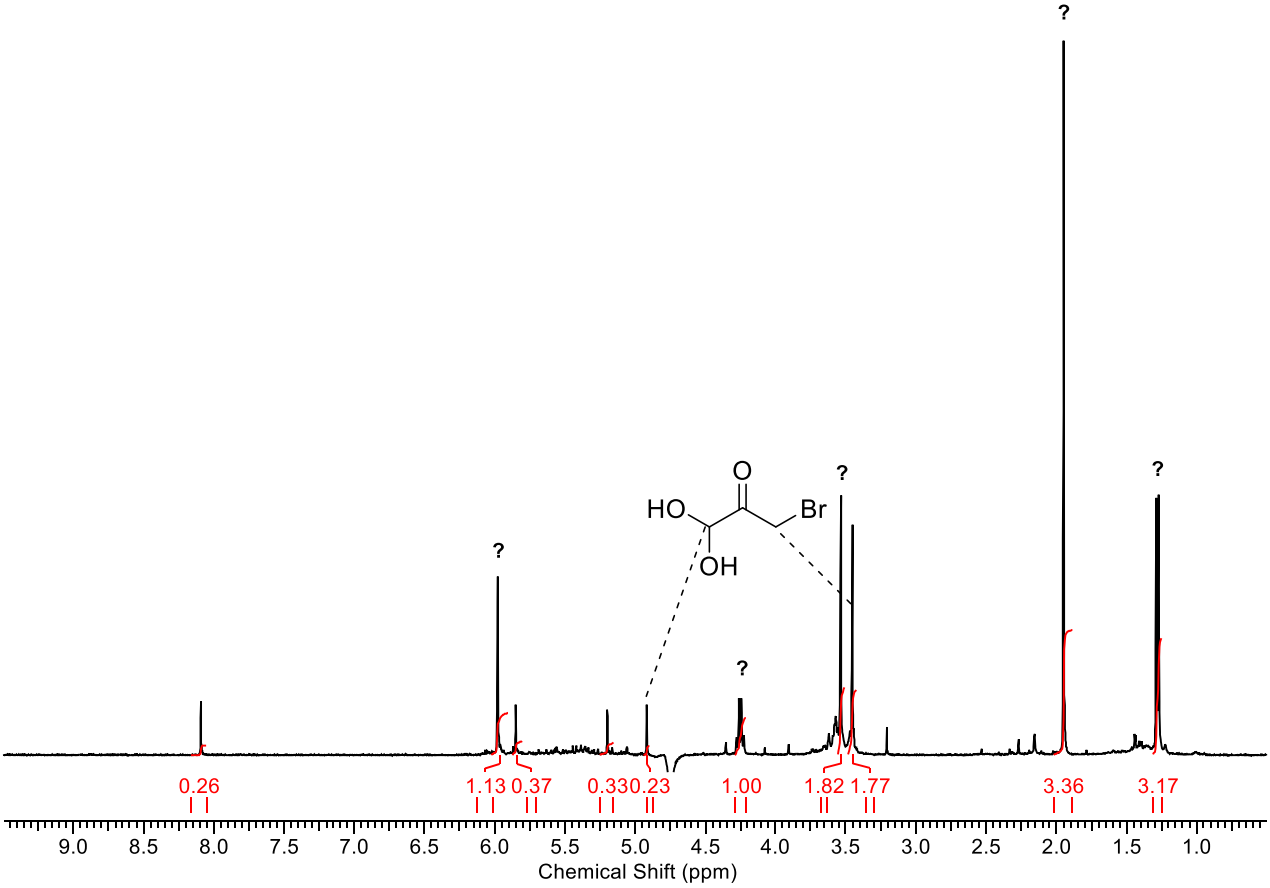


A45: IR spectrum of **108**.

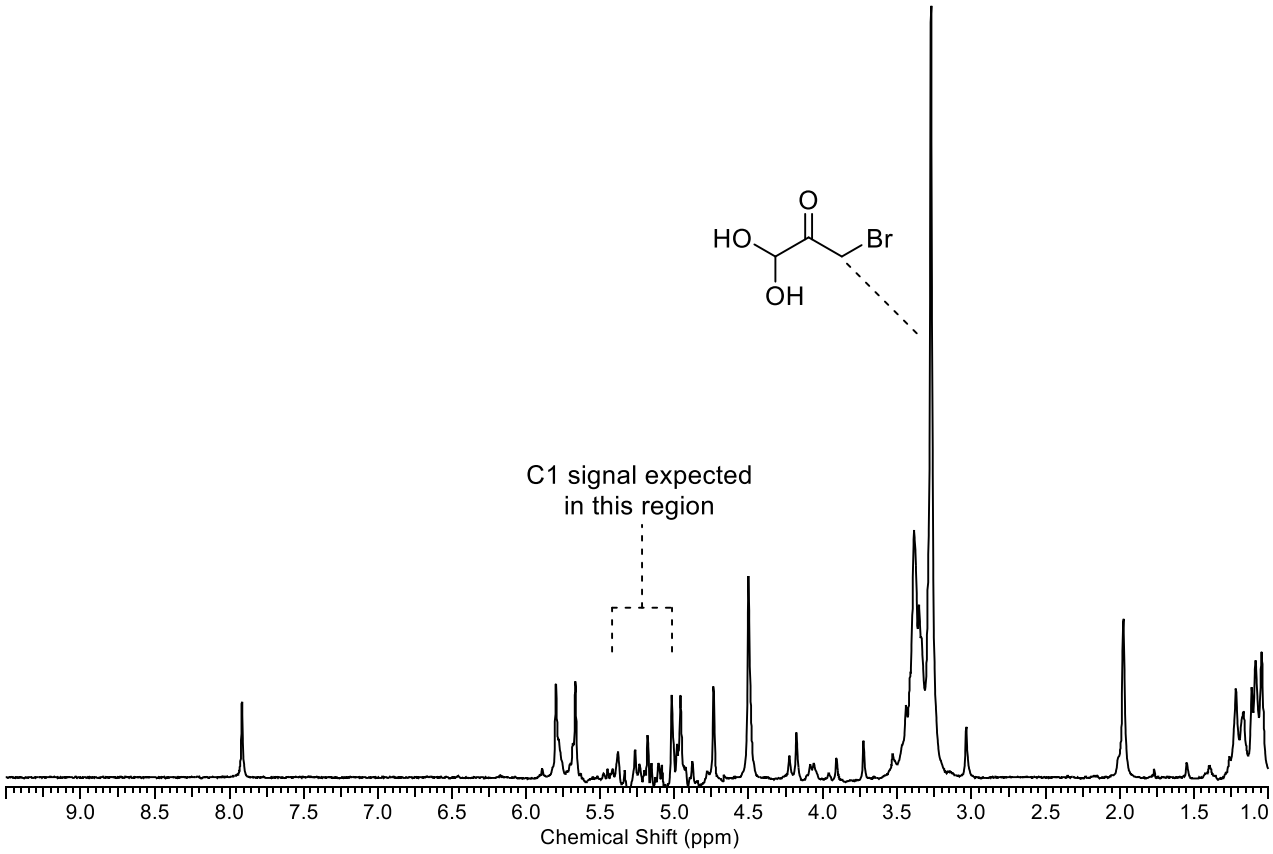


Bromomethylglyoxal

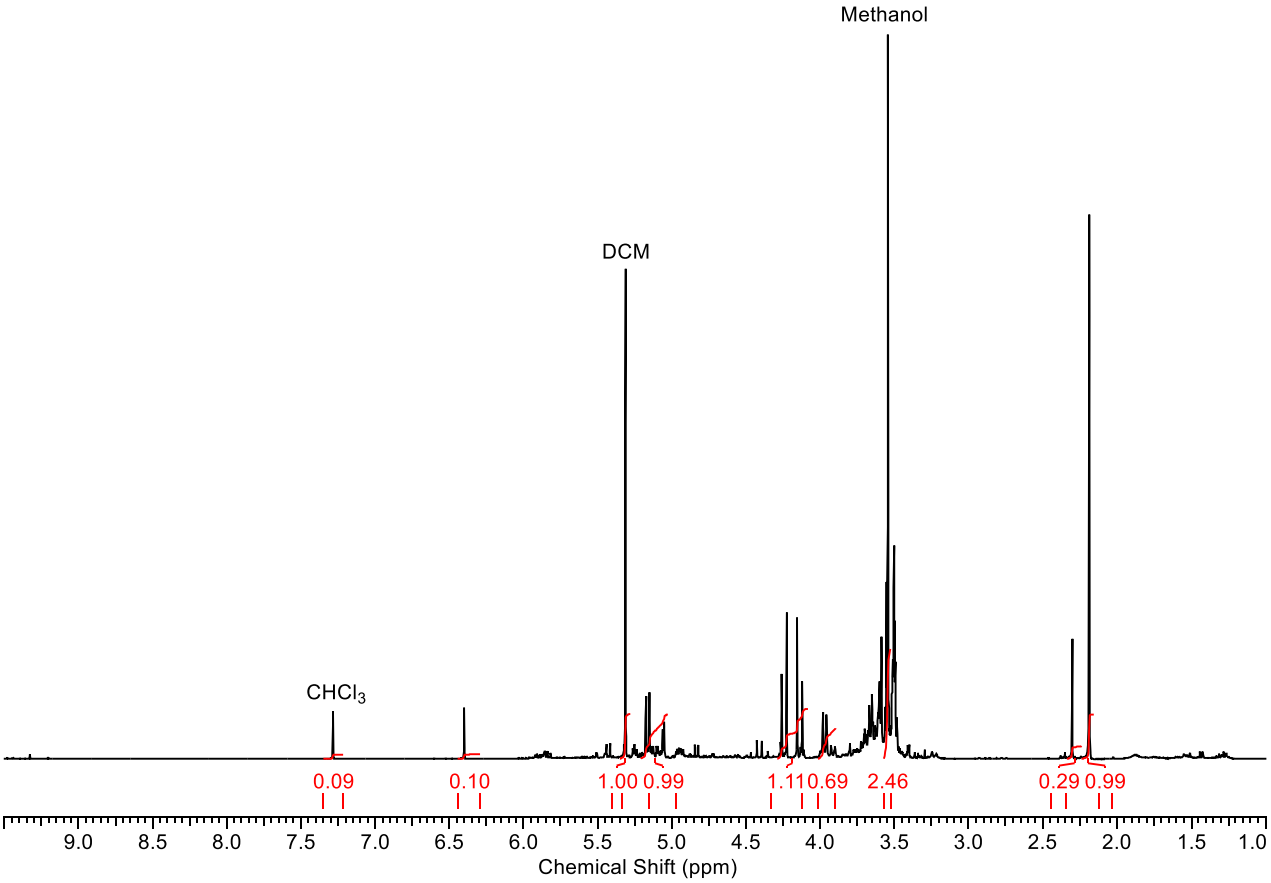
A46: ^{1}H NMR spectrum (400 MHz, {H_2O/D_2O, 9:1}, 1.0 – 9.5 ppm) of the reaction of methylglyoxal (**75**, 2.8M) with bromine (1.7 eq.) in the presence of catalytic H_2SO_4 at 75 °C, 3 h. Peaks corresponding to bromomethylglyoxal are tentatively assigned.



A47: ^{1}H NMR spectrum (300 MHz, {H_2O/D_2O, 9:1}, 1.0 – 9.5 ppm) with dual suppression of water and acetic acid peaks of the reaction of methylglyoxal (**75**, 2.3M) with bromine (1.1 eq.) in the presence of acetic acid (0.9 eq.) at 75 °C, 3 h with dual suppression of water and acetic acid. The peak corresponding to the C3 position of bromomethylglyoxal is tentatively assigned, with the C1 signal assumed to be supressed with the water peak.

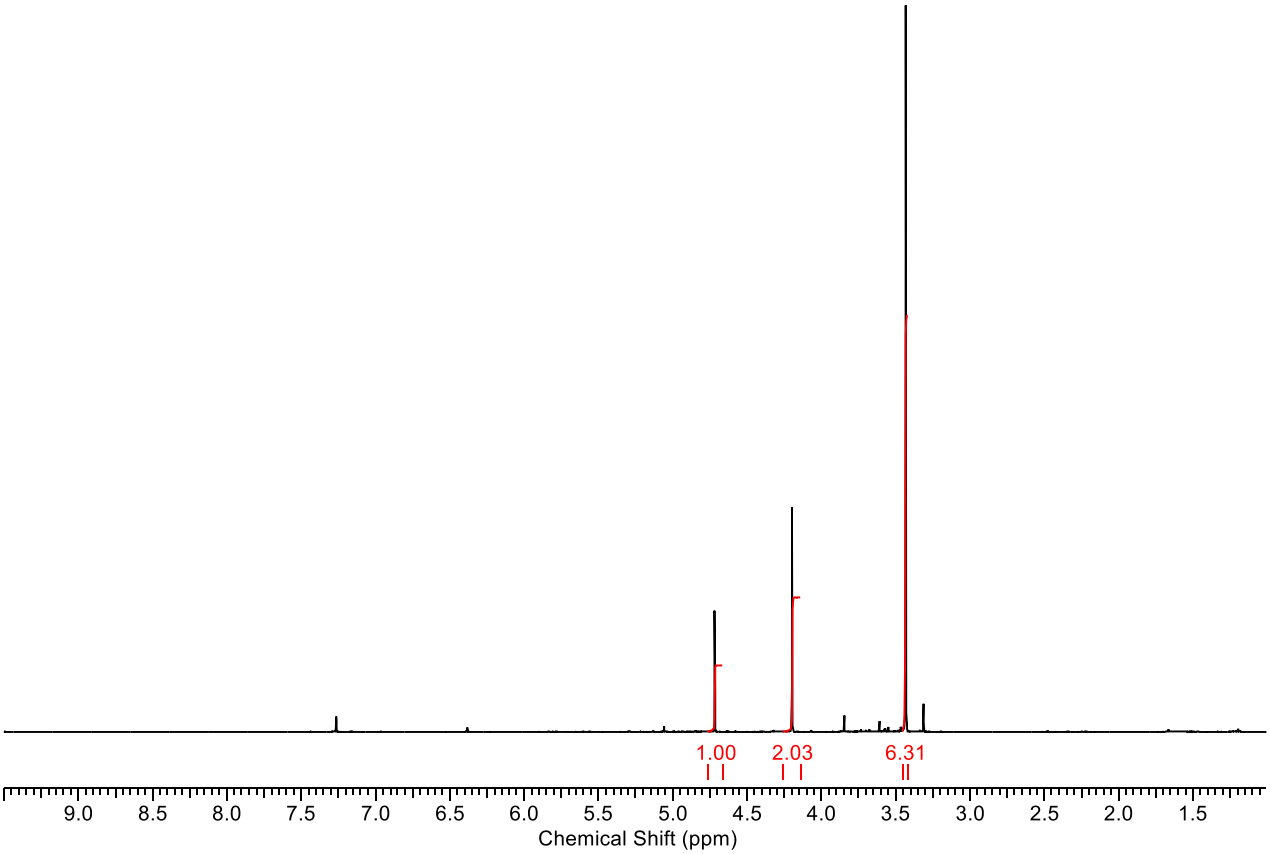


A48: ^{1}H NMR spectrum (400 MHz, {$CDCl_3$}, 1.0 – 9.5 ppm) with dual suppression of water and acetic acid peaks of the reaction of methylglyoxal (**75**, 2.3M) with bromine (1.1 eq.) in the presence of acetic acid (0.9 eq.) at 75 °C, 1.2 h after attempted purification by flash chromatography (DCM/MeOH, 50:1).

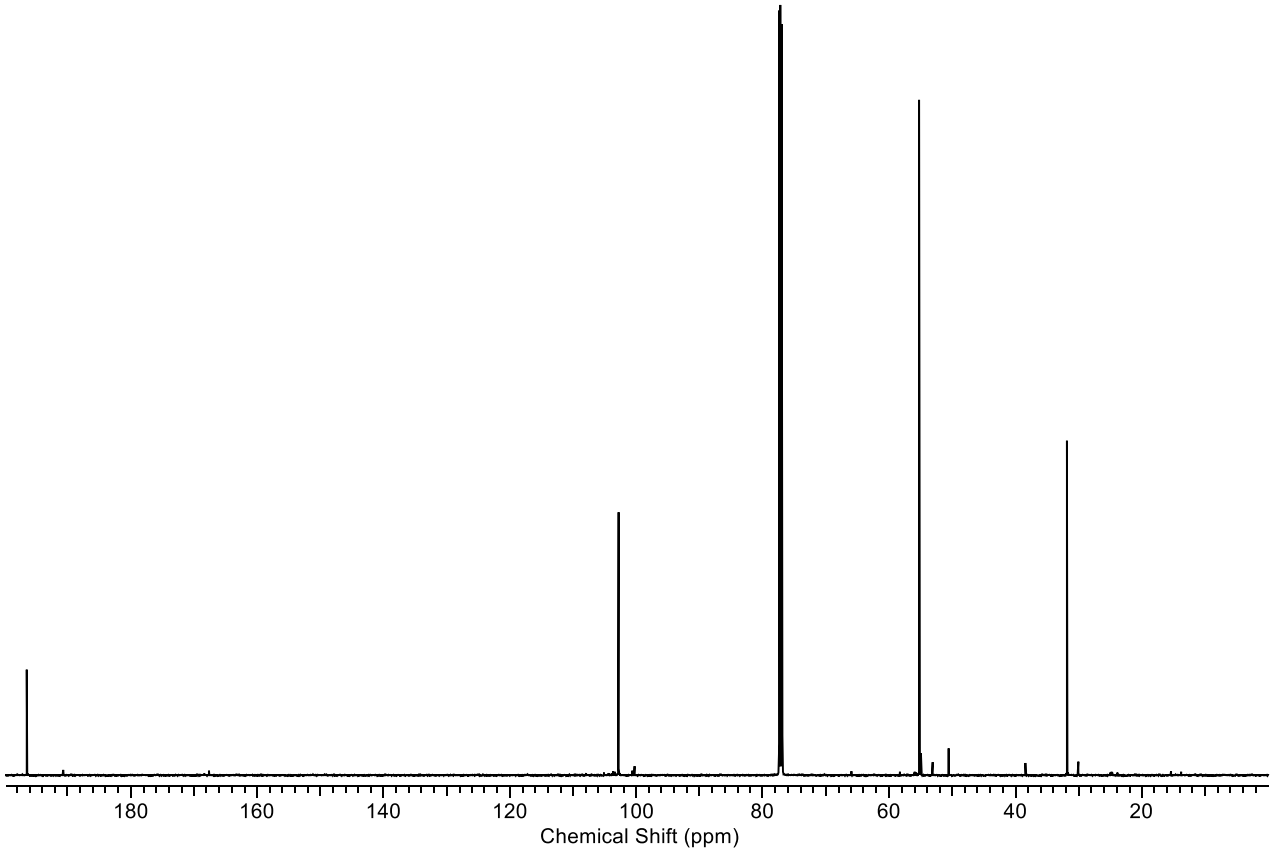


Bromomethylglyoxal dimethyl acetal (**111**)

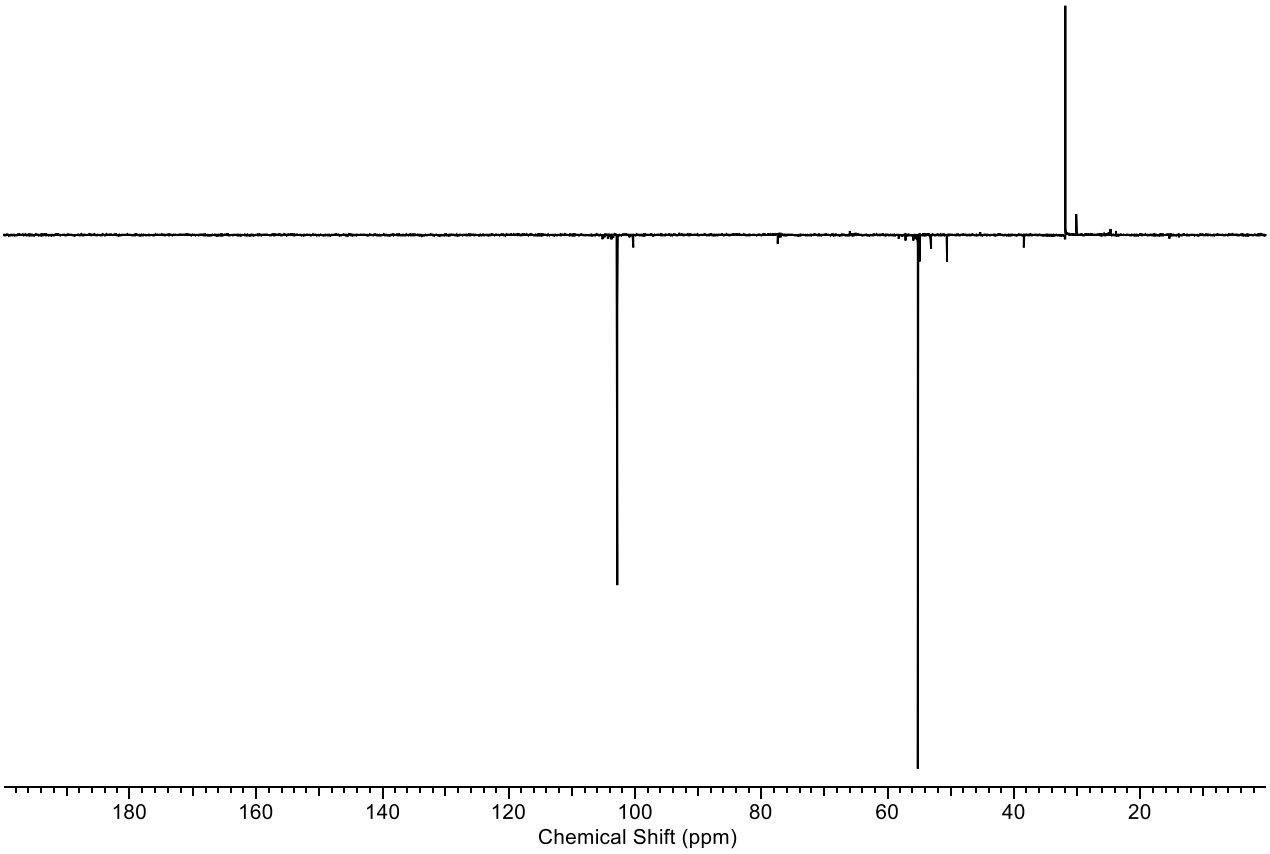
A49: ^{1}H NMR spectrum (600 MHz, {$CDCl_3$}, 1.0 – 9.5 ppm) of bromomethylglyoxal dimethyl acetal (**111**).



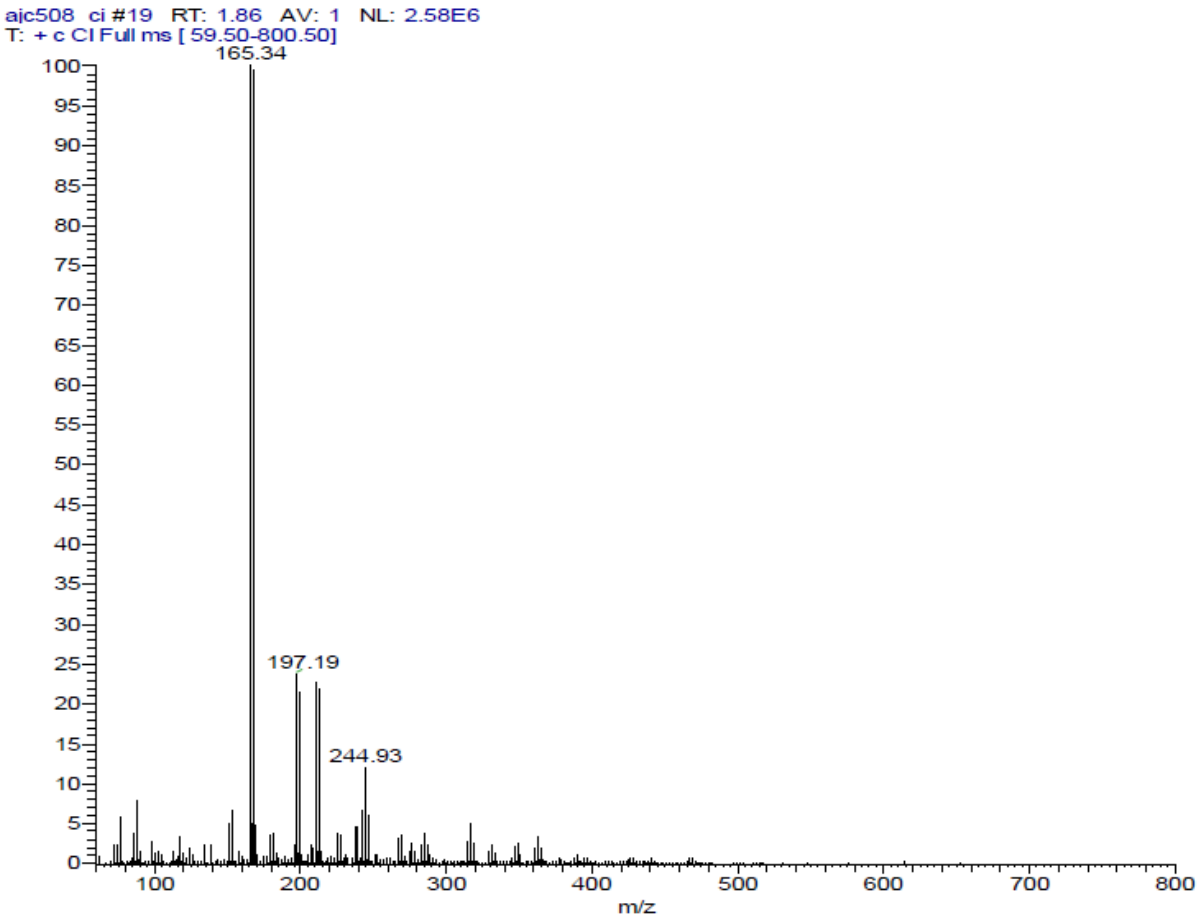
A50: ^{13}C NMR spectrum (151 MHz, {$CDCl_3$}, 0 – 200 ppm) of **111**.

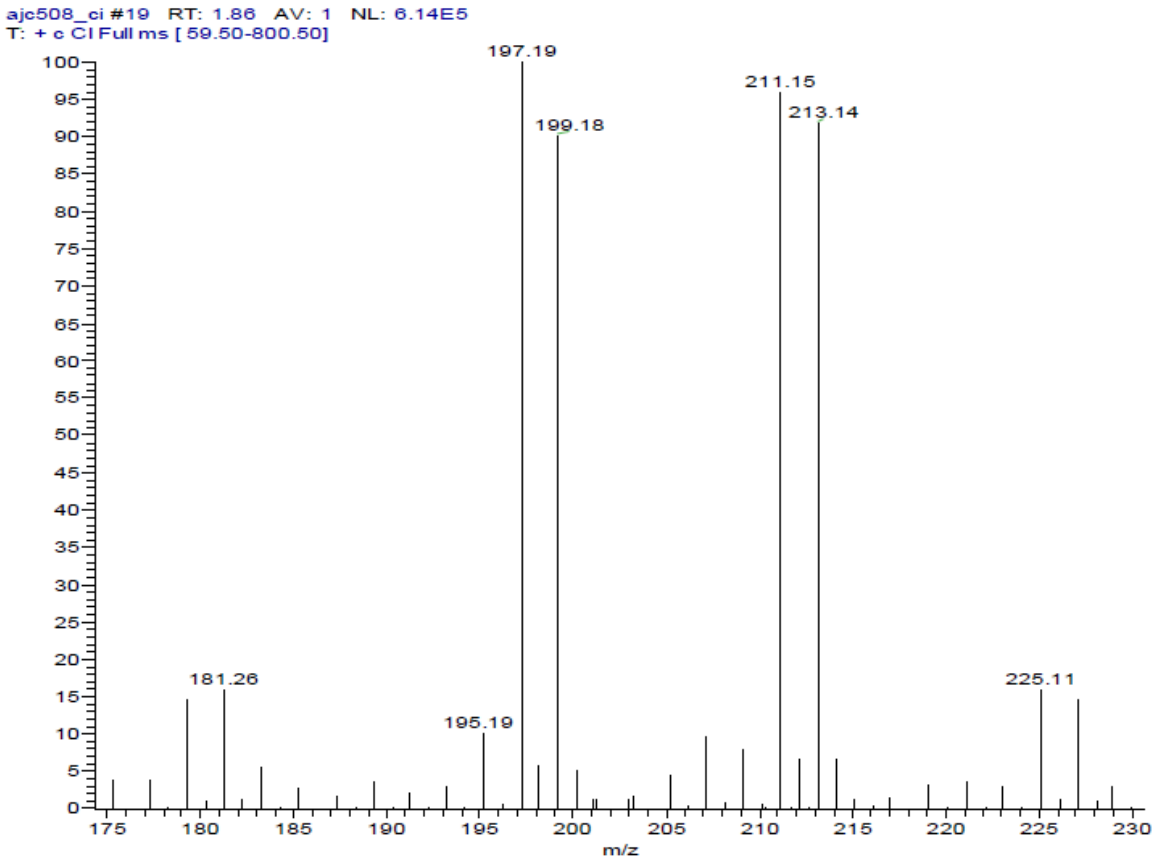


A51: ^{13}C DEPT 135 NMR spectrum (151 MHz, {$CDCl_3$}, 0 – 200 ppm) of **111**.

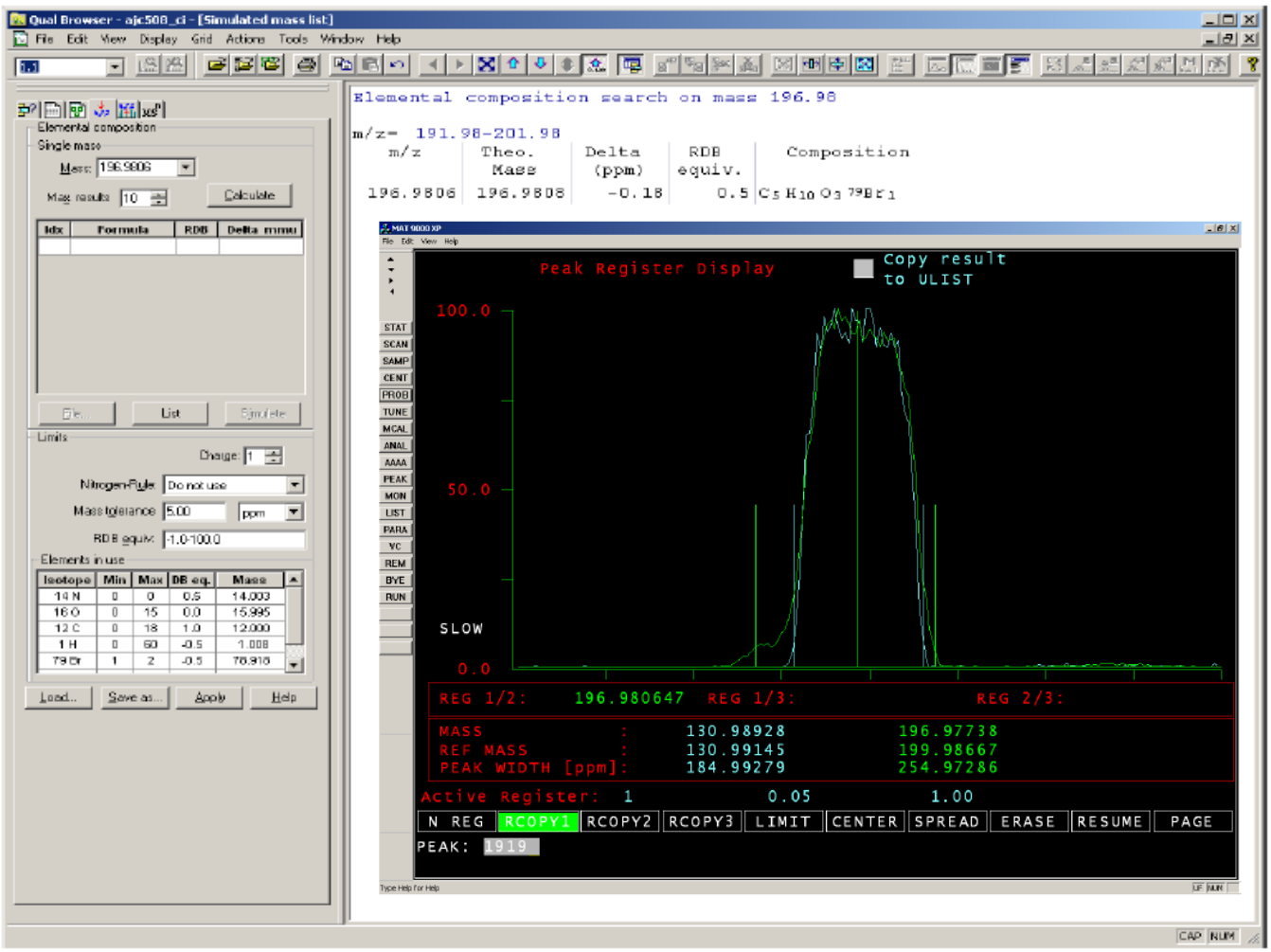


A52: **Top**, CI+ mass spectrum of **111**. **Bottom**, expansion.

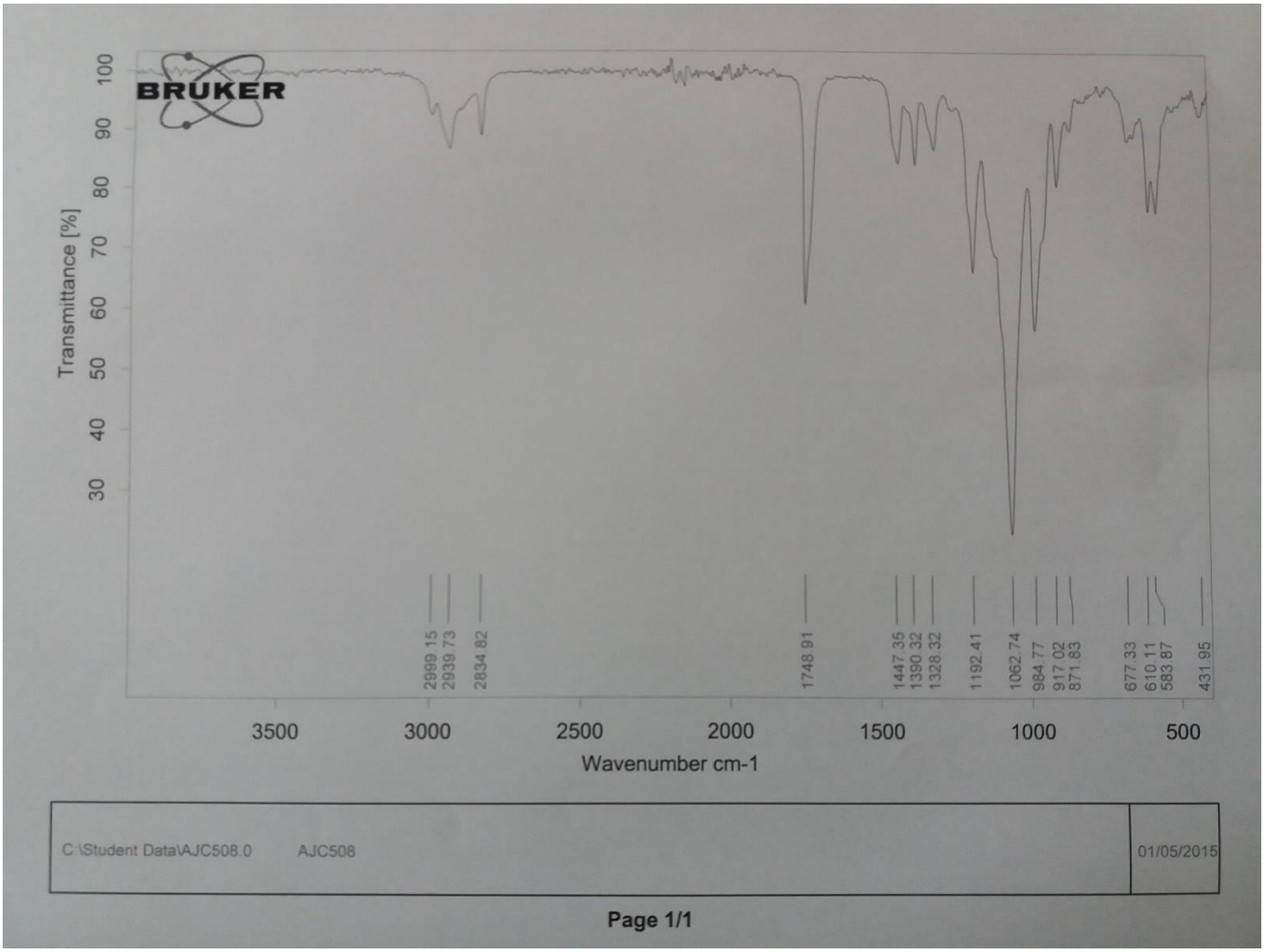




A53: HRMS of **111**.

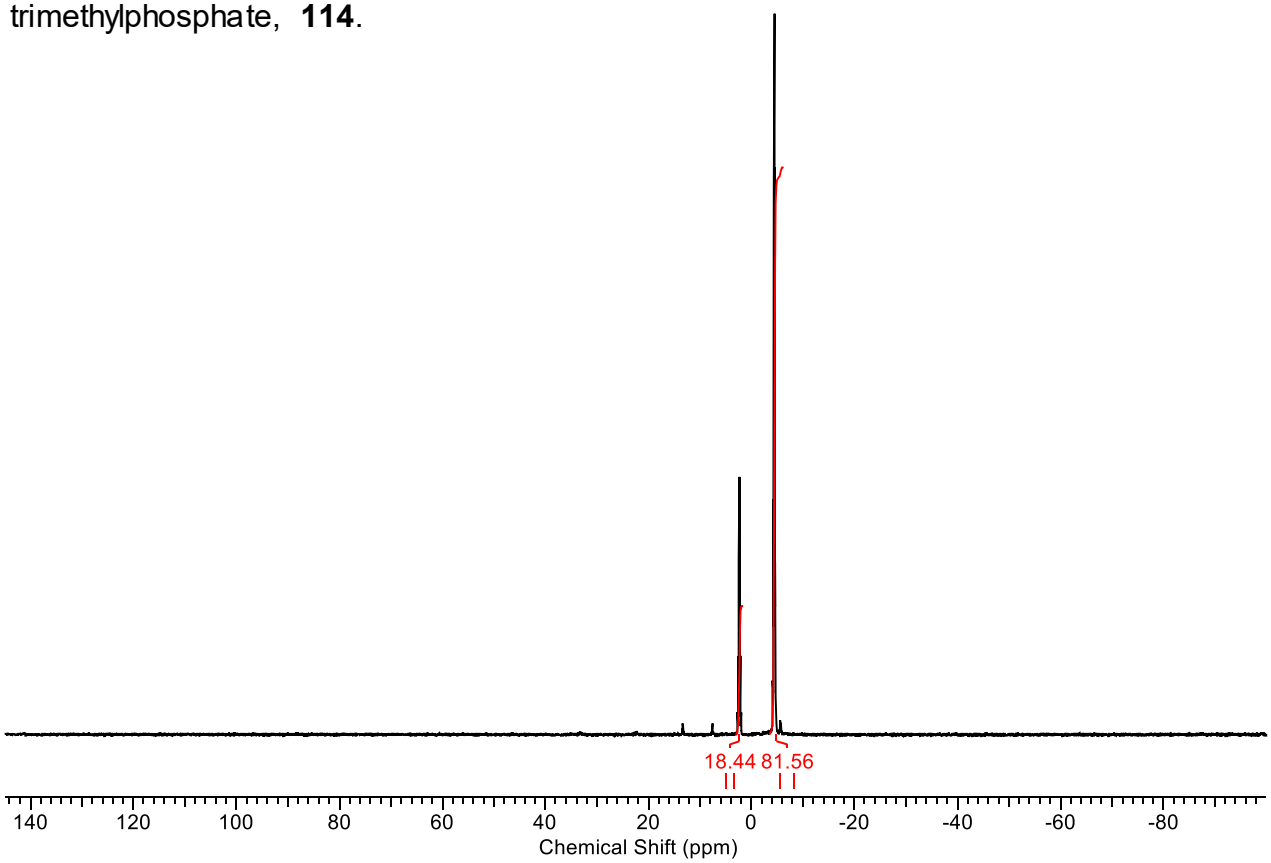


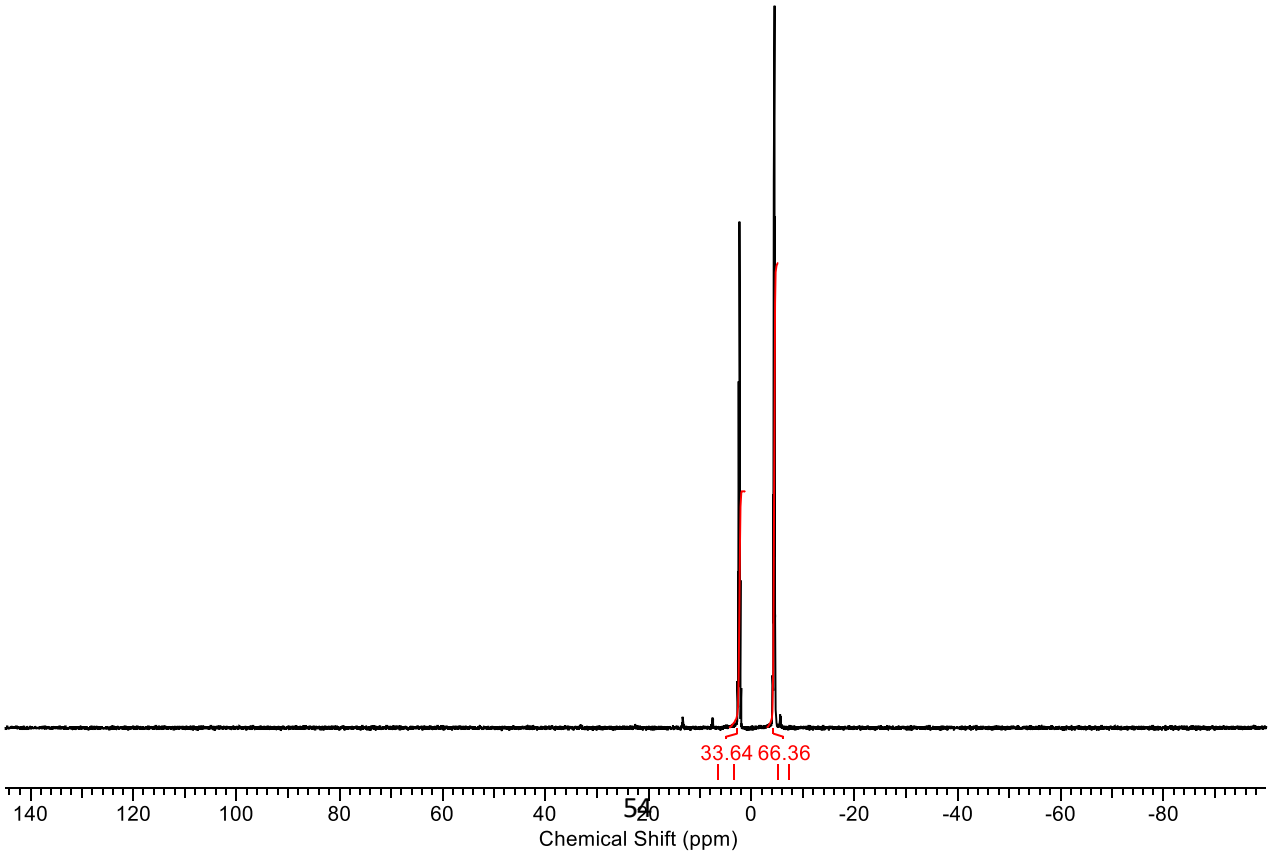
A54: IR spectrum of **111**.



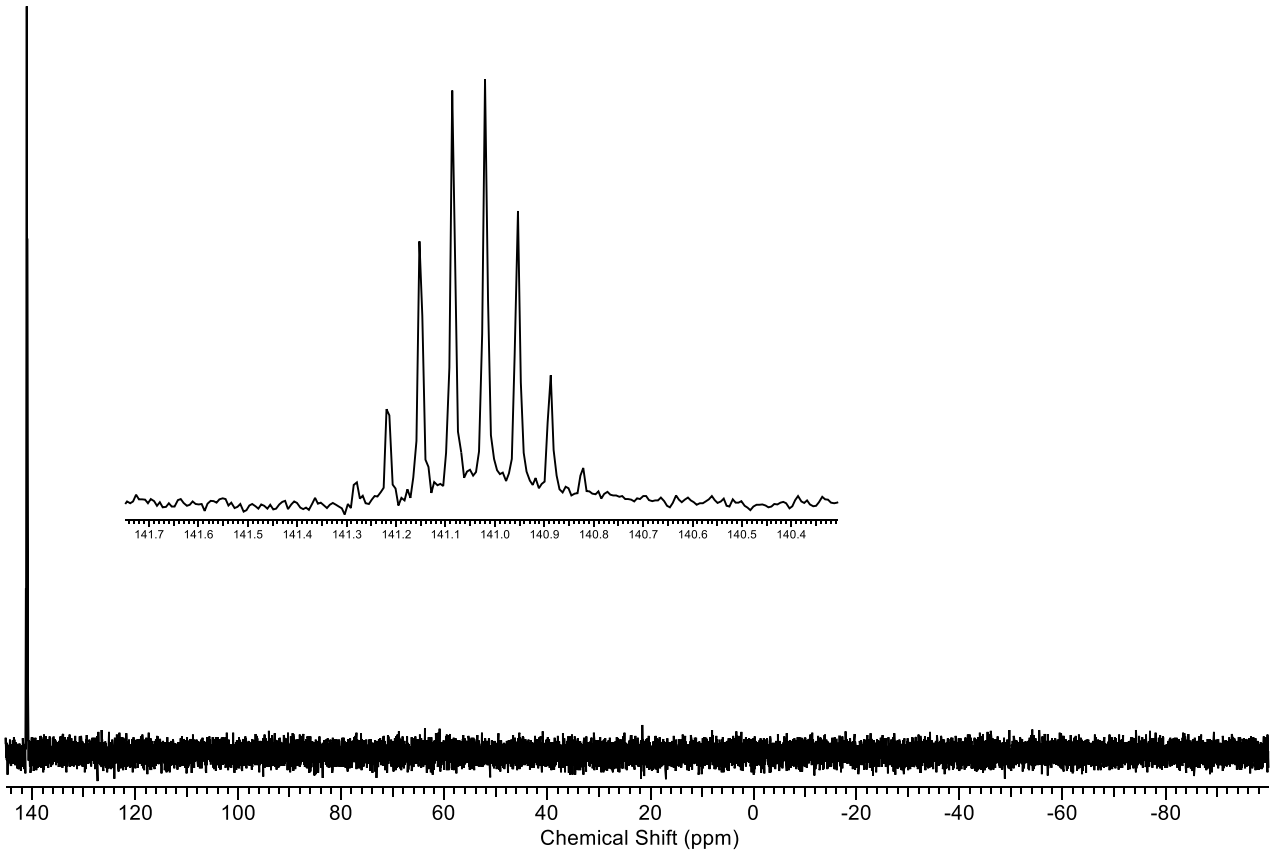
(Dimethyl)phosphoenolpyruvaldehyde dimethyl acetal, **113**

A55: ^{31}P NMR spectra (121 MHz, {$CDCl_3$}, -100 – 145 ppm) of **Top**, the reaction of bromomethylglyoxal dimethyl acetal, (**111**, 86mM) with trimethylphosphite (1.1 eq) in diethyl ether at ambient temperature overnight after evaporation and **Bottom**, the same sample after spiking with pure trimethylphosphate, **114**.

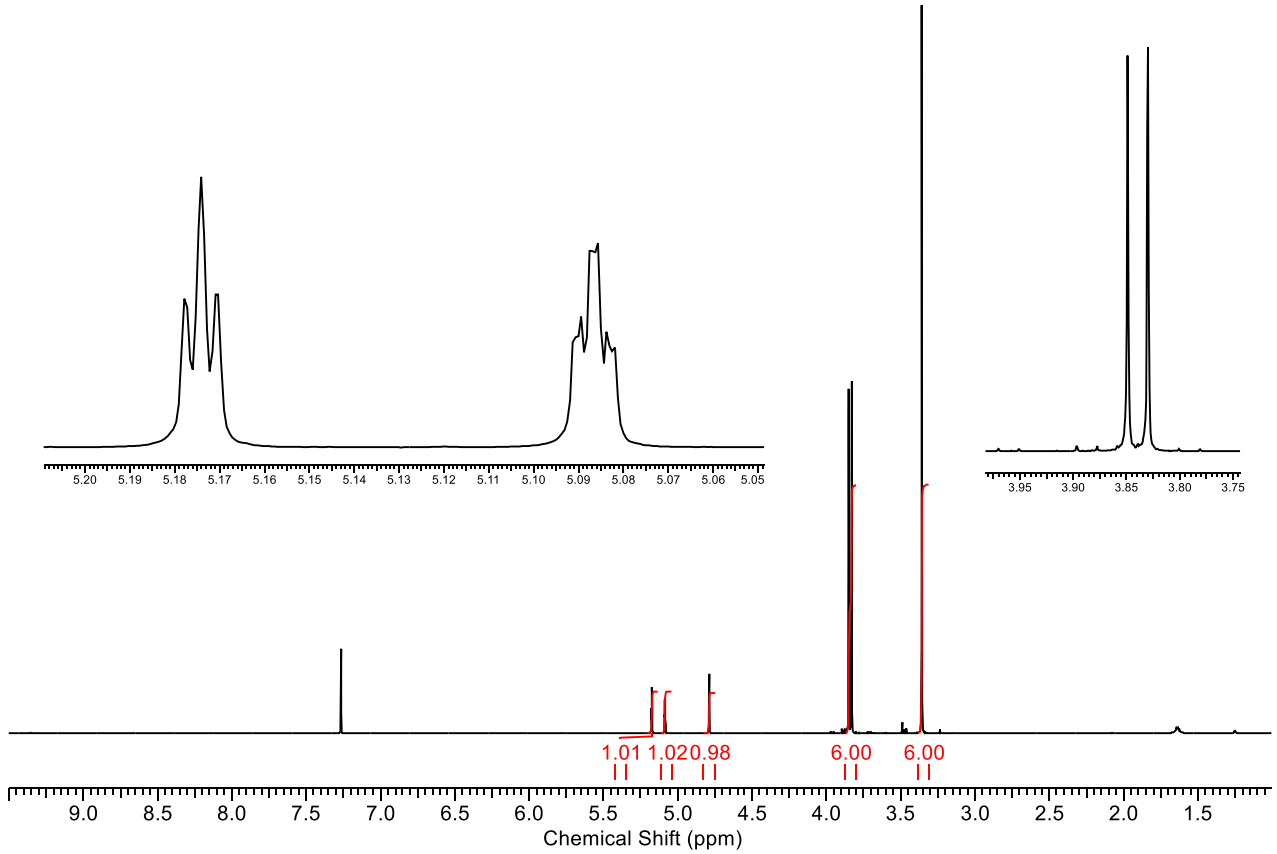




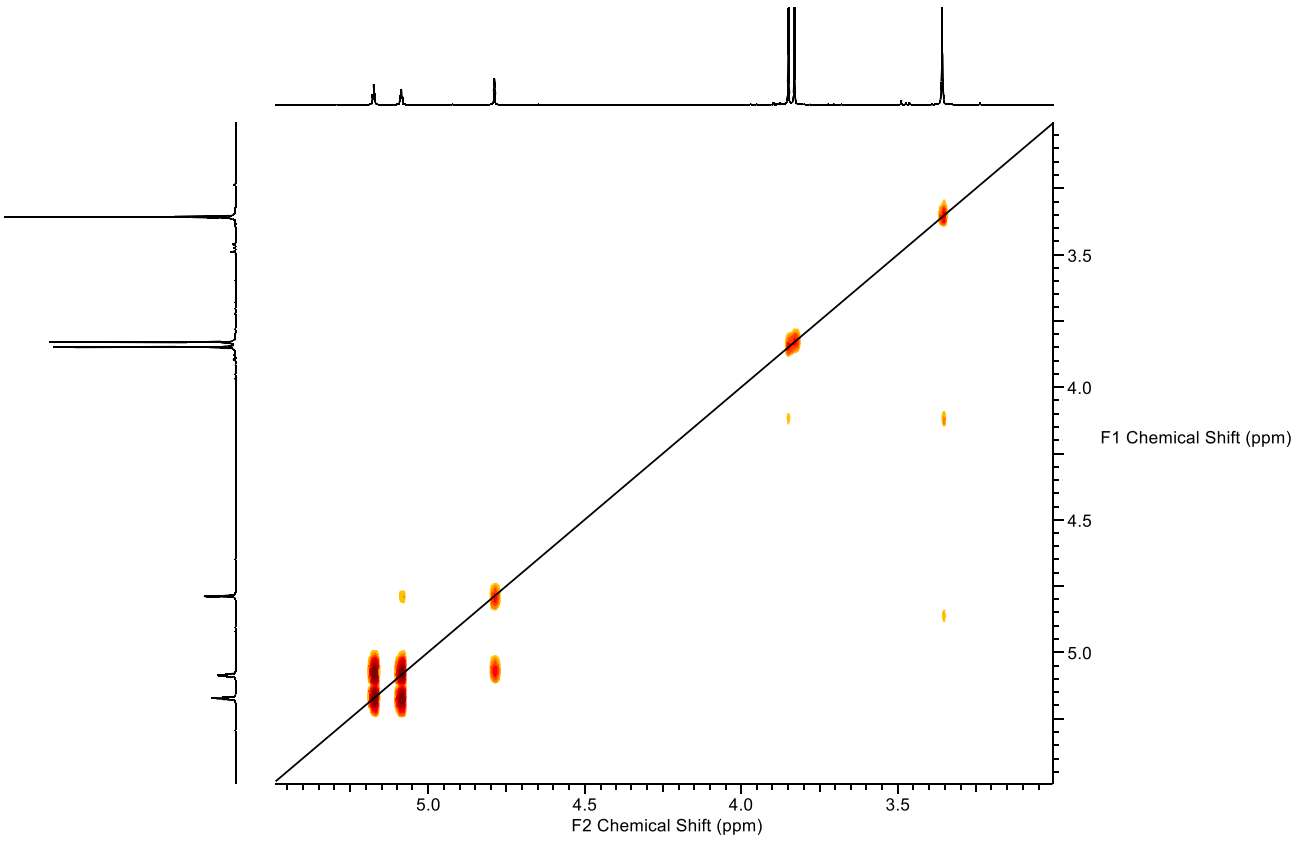
A56: ^{31}P NMR spectrum (162 MHz, {$CDCl_3$}, -100 – 145 ppm) of trimethylphosphite after incubation at ambient temperature in diethyl ether in air for 20 h with expansion overlaid. The signal is a decet (ten lines) due to coupling to the protons of three methoxyl groups, however the outermost peaks are lost to baseline noise.



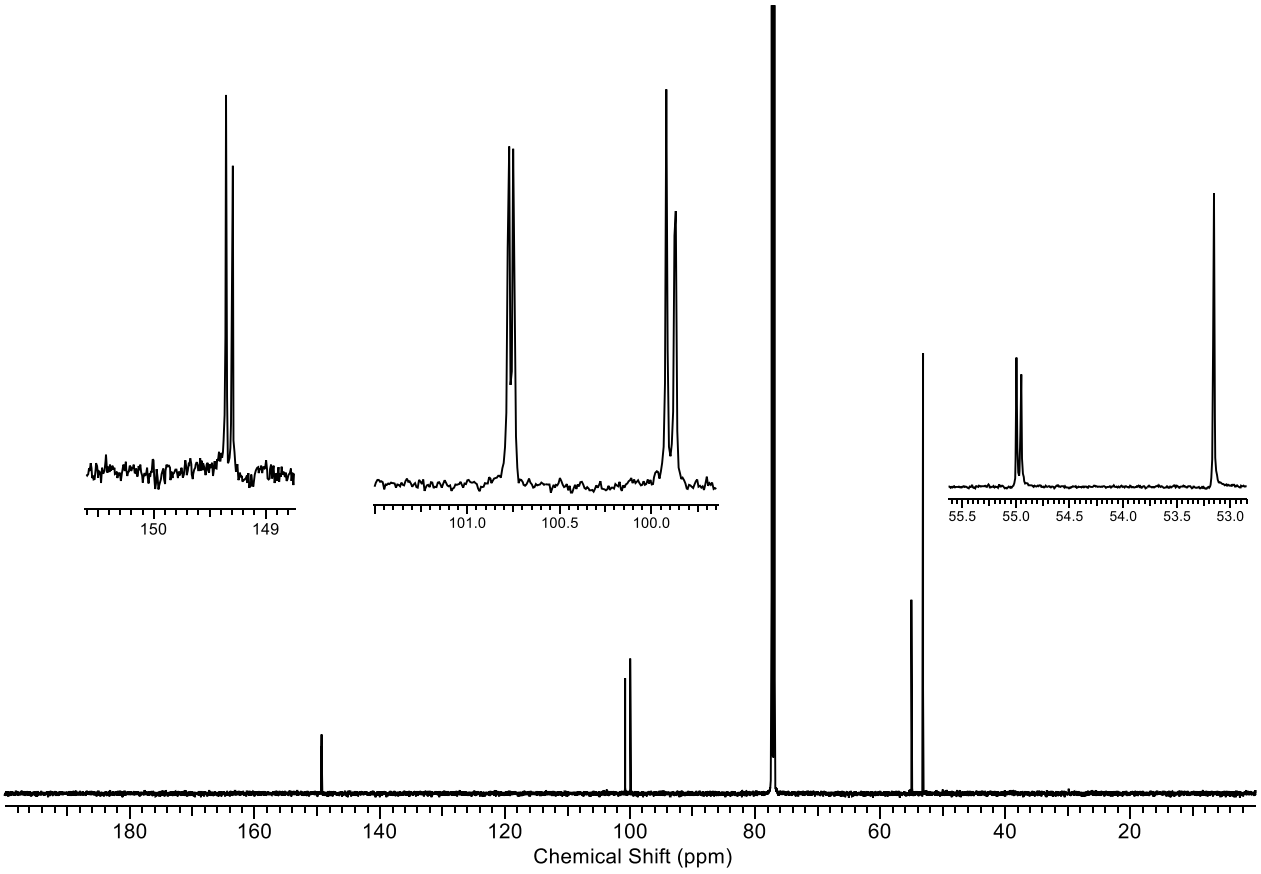
A57: ^{1}H NMR spectrum (600 MHz, {$CDCl_3$}, 1.0 – 9.5 ppm) of (dimethyl)phosphoenolpyruvaldehyde dimethyl acetal (**113**) with expansions overlaid.



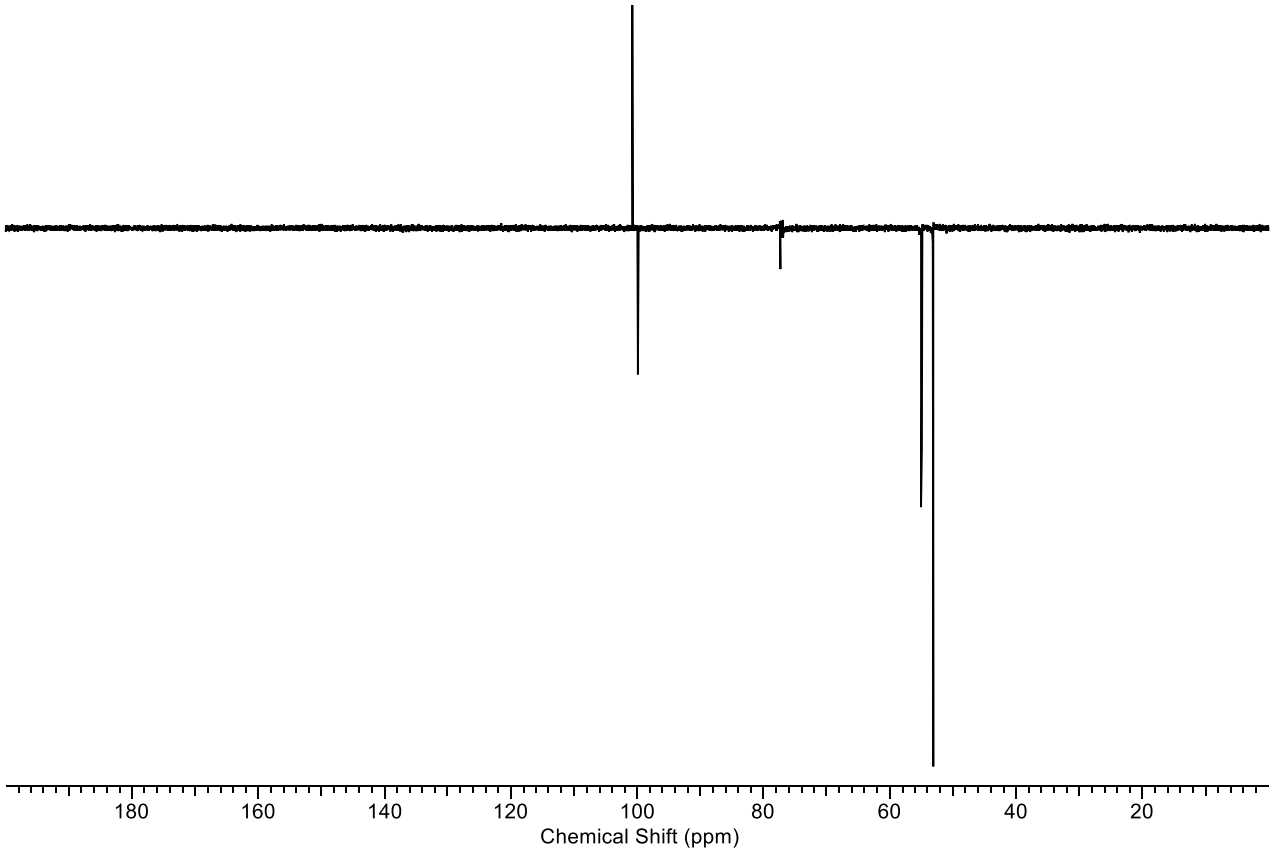
A58: ^{1}H-^{1}H COSY NMR spectrum (600 MHz, {$CDCl_3$}) of **113**.



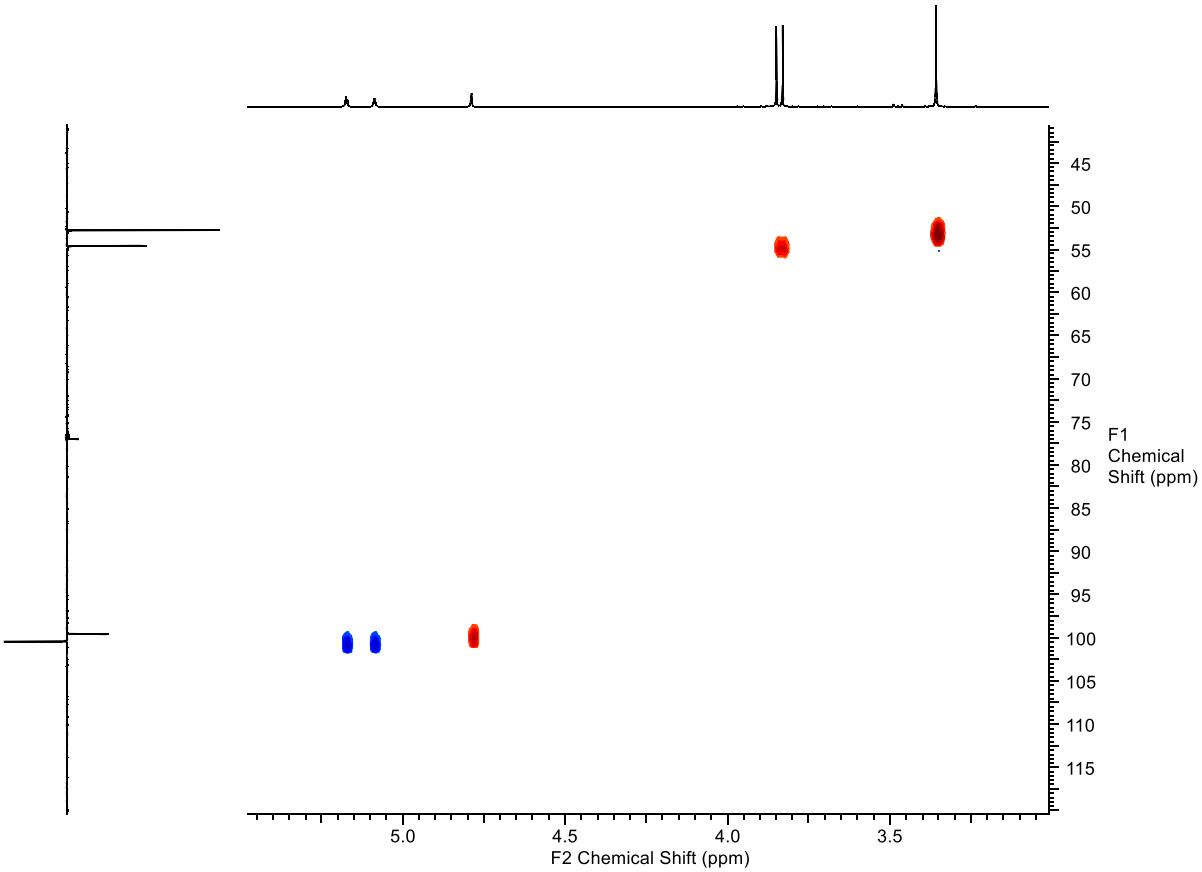
A59: ^{13}C NMR spectrum (151 MHz, {$CDCl_3$}, 0 – 200 ppm) of **113** with expansions overlaid.



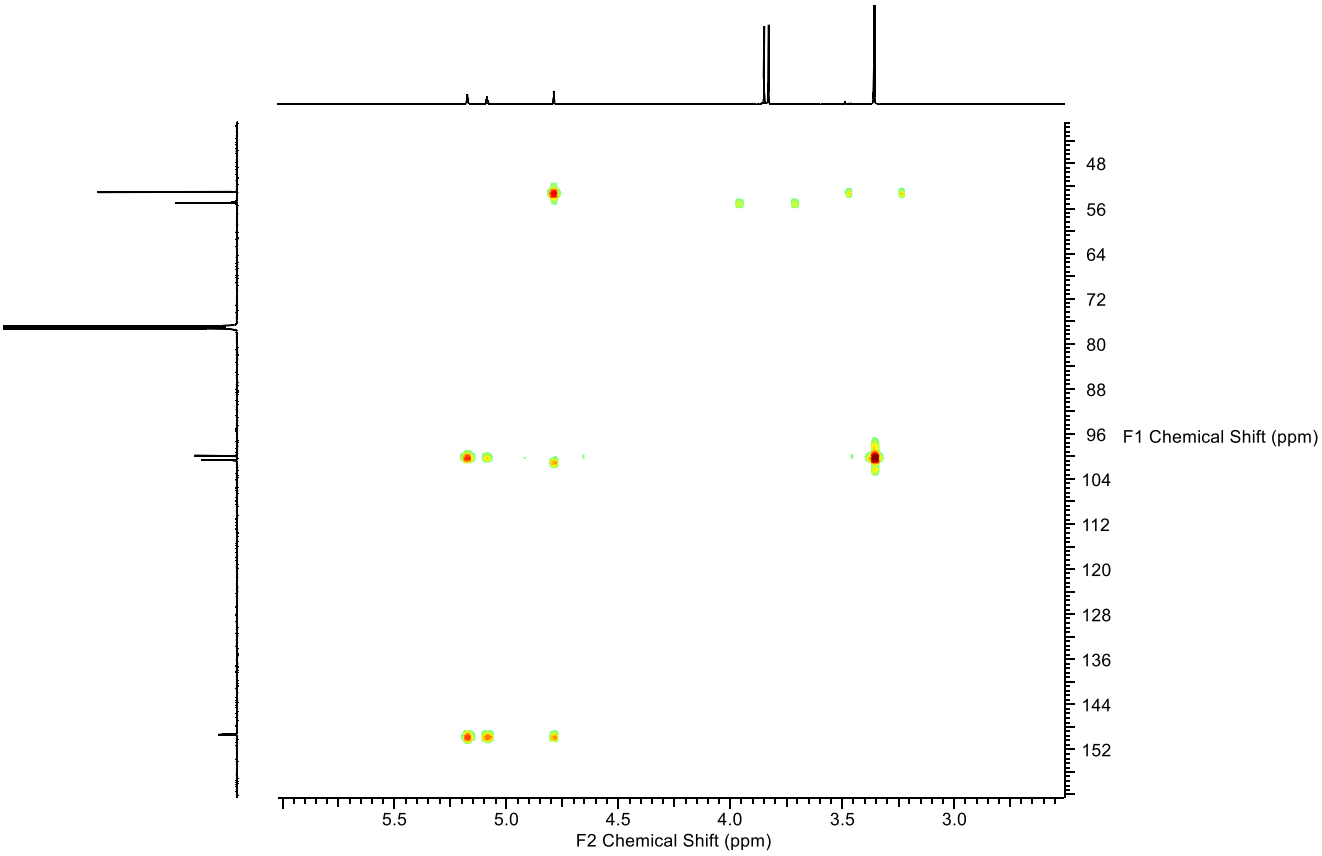
A60: ^{13}C DEPT 135 NMR spectrum (151 MHz, {$CDCl_3$}, 0 – 200 ppm) of **113**.



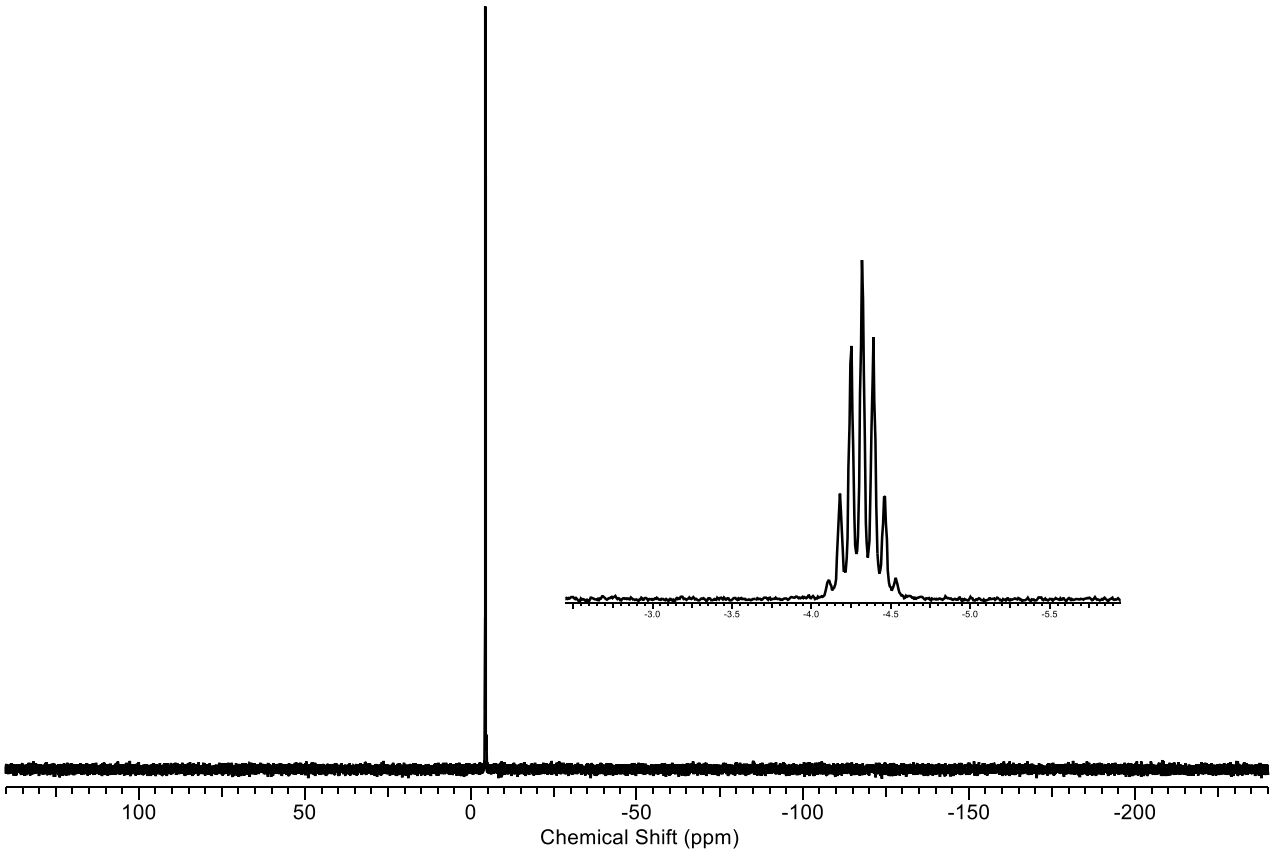
A61: ^{1}H-^{13}C HSQC NMR spectrum (600 MHz, {$CDCl_3$}) of **113**.



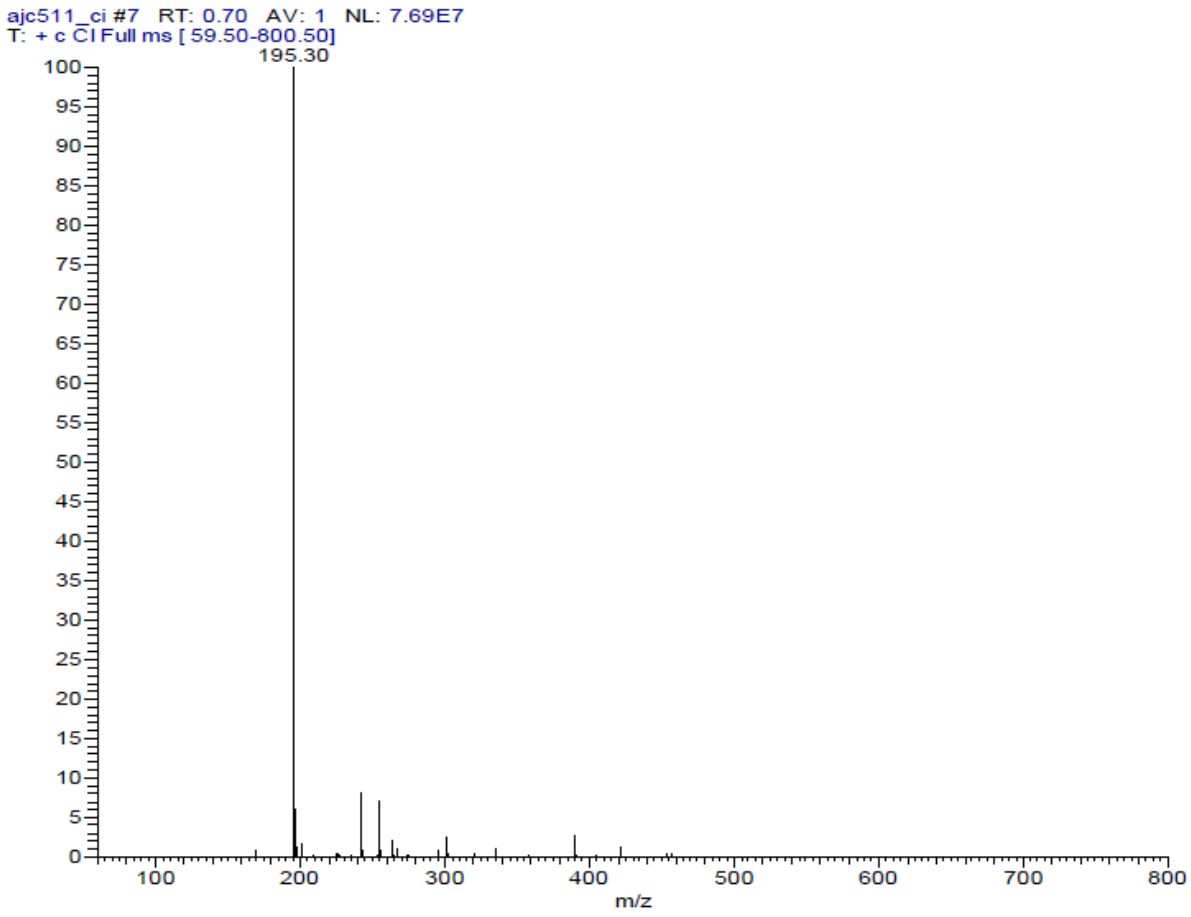
A62: ^{1}H-^{13}C HMBC NMR spectrum (600 MHz, {$CDCl_3$}) of **113**.



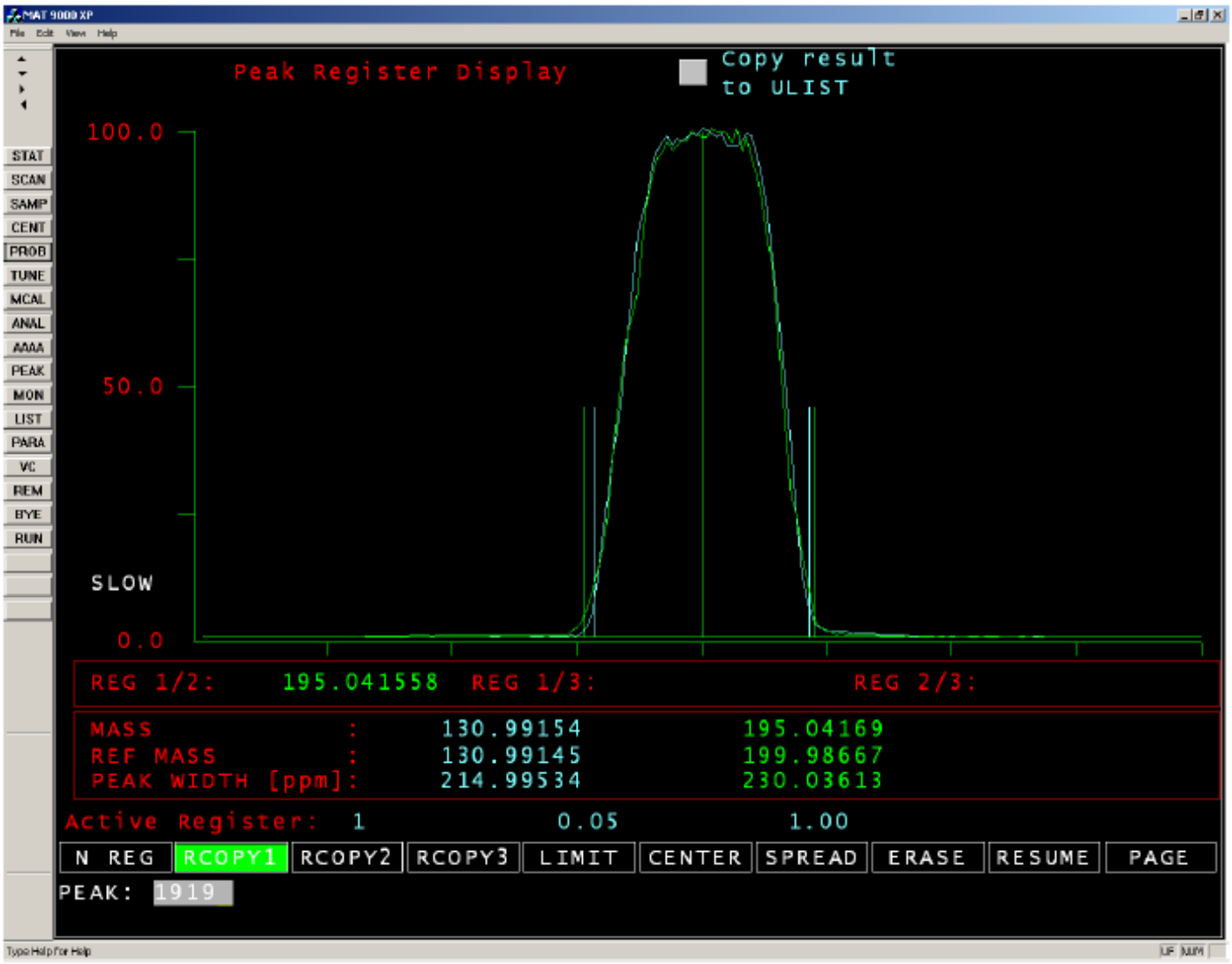
A63: 31P NMR spectrum (162 MHz, {$CDCl_3$}, -240 – 140 ppm) of **113** with expansion (-6.0 – -2.5 ppm) overlaid.

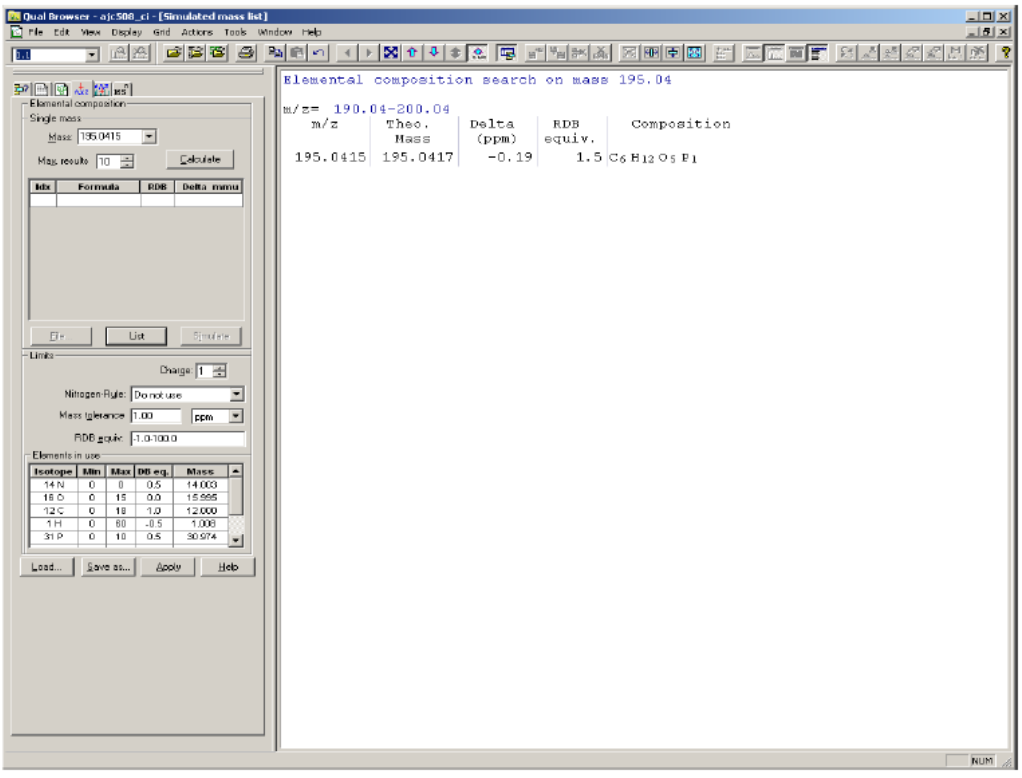


A64: CI+ mass spectrum of **113**. The peak at 195 is due to loss of OMe from the molecular ion.

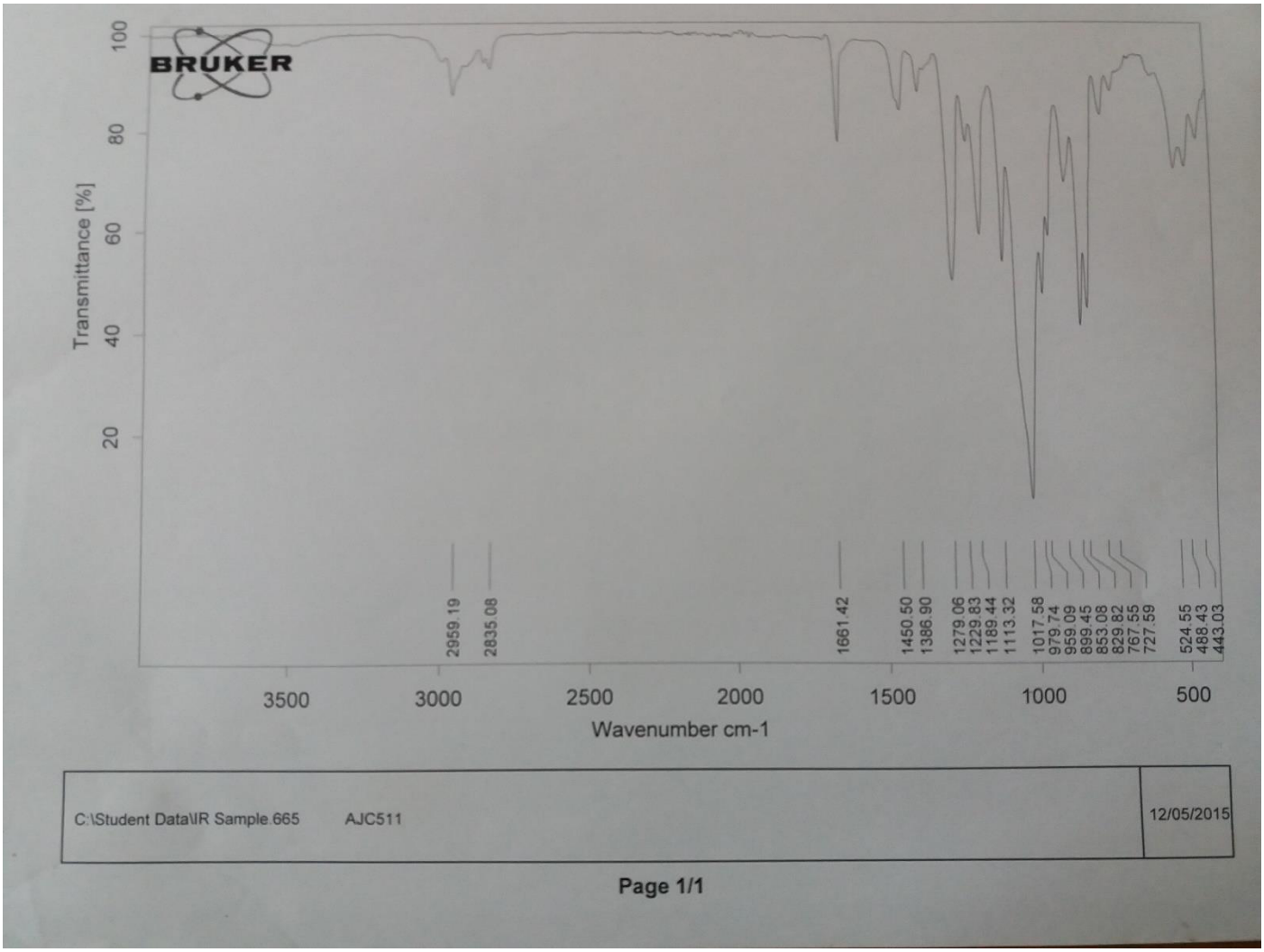


A65: HRMS of **113**. Accurate mass data taken for the peak due to the molecular ion minus OMe.



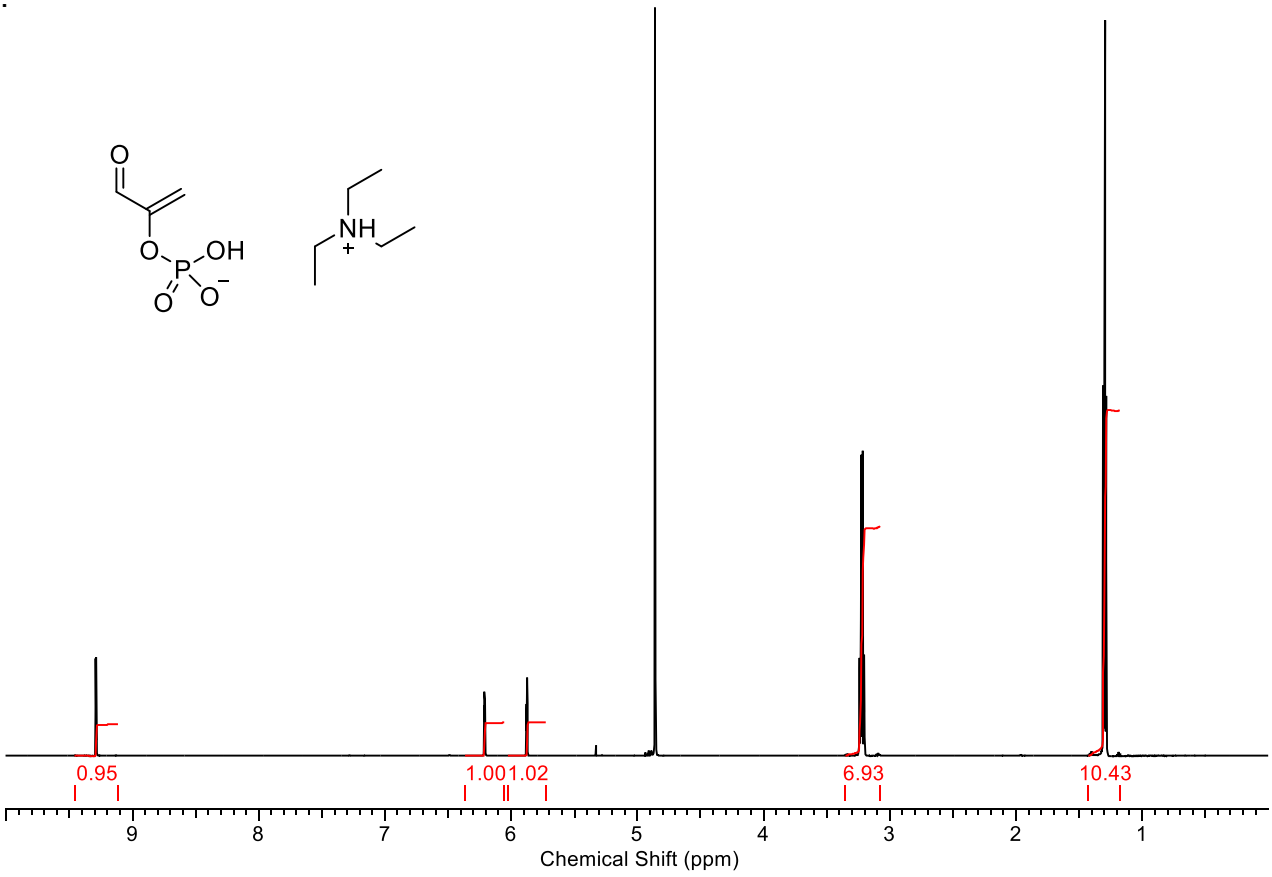


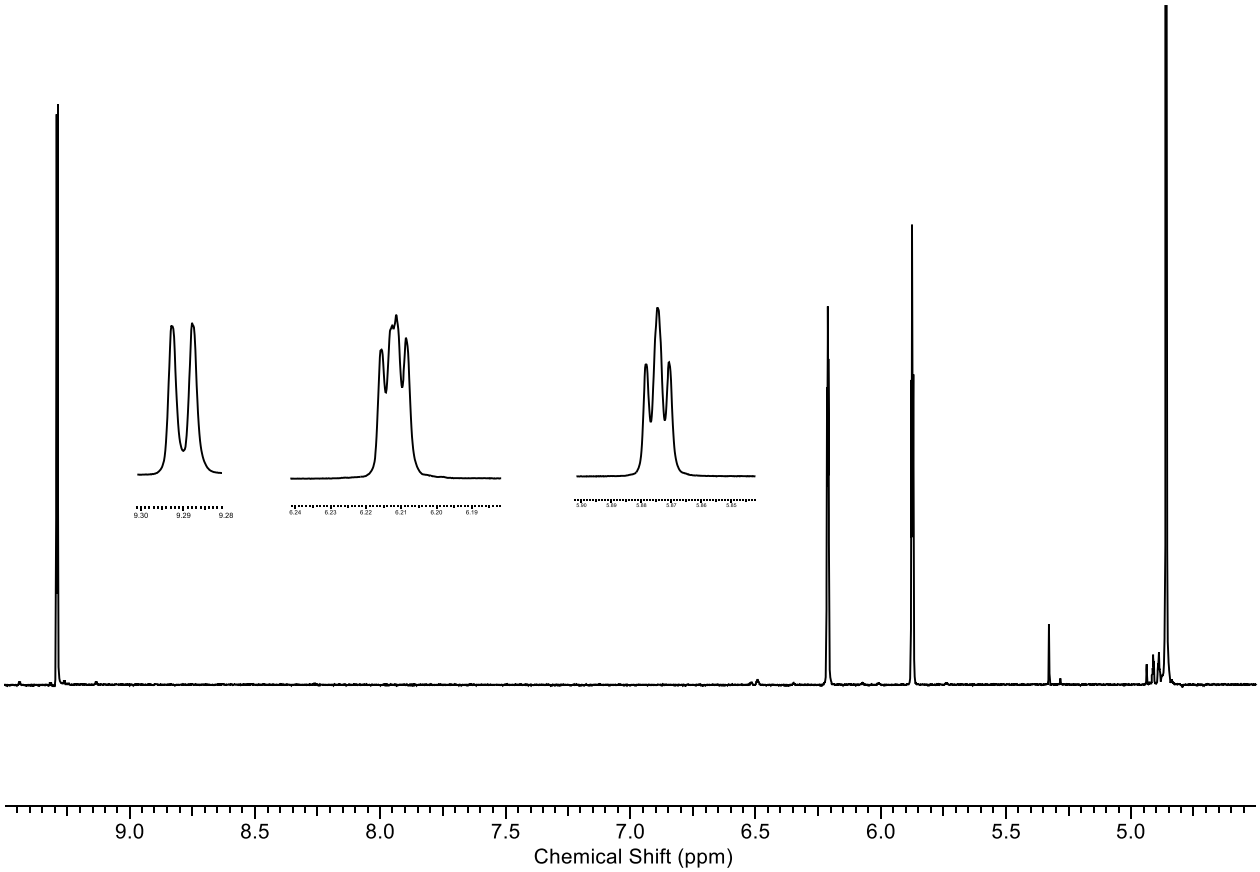
A66: IR spectrum of **113**.



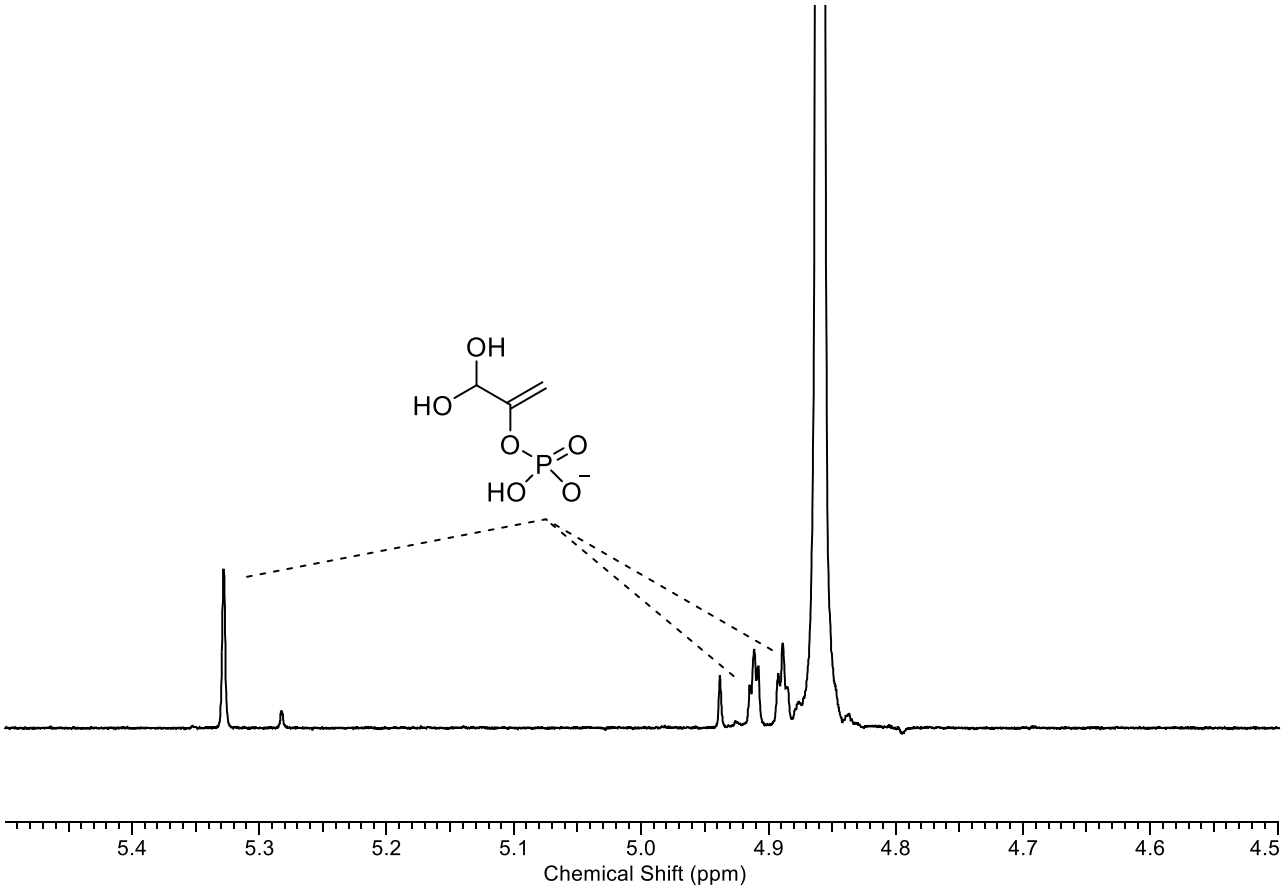
Phosphoenolpyruvaldehyde, **105**

A67: ^{1}H NMR spectrum (600 MHz, {D_2O}, 0.0 – 9.5 ppm) of phosphoenolpyruvaldehyde, **105**, triethylammonium salt with expansion (4.5 – 9.5 ppm, with individual peak expansions overlaid) below.

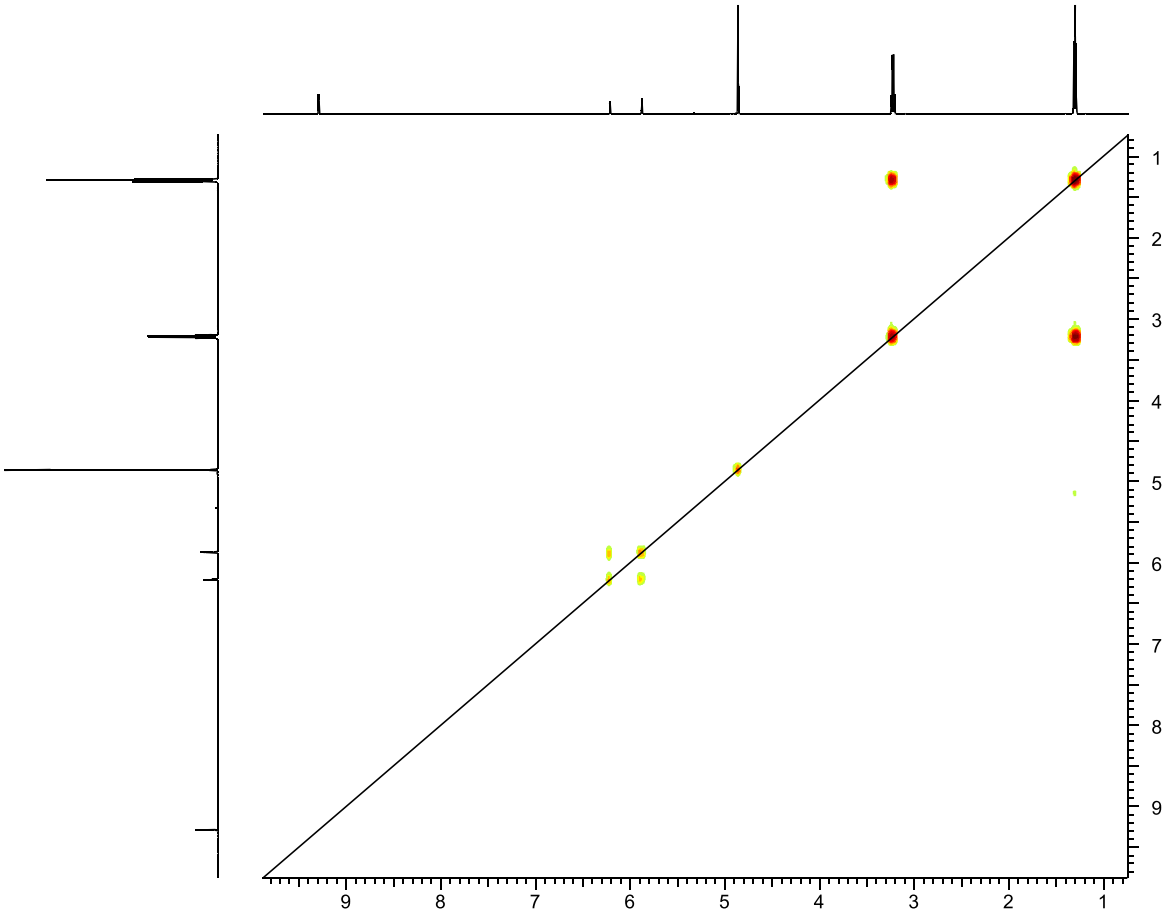


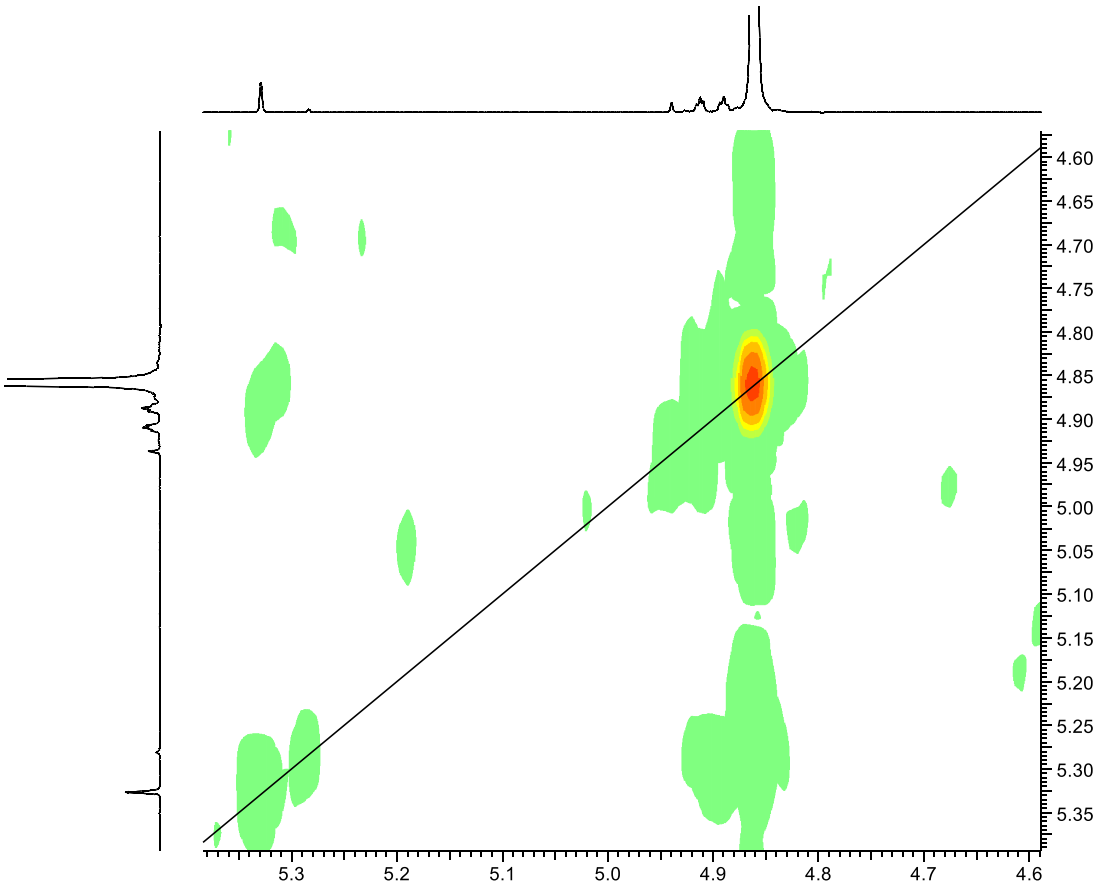


A68: Further expanded ^{1}H NMR spectrum (600 MHz, {D_2O}, 4.5 – 5.5 ppm) of **105** triethylammonium salt, showing peaks for the minor, hydrated form.

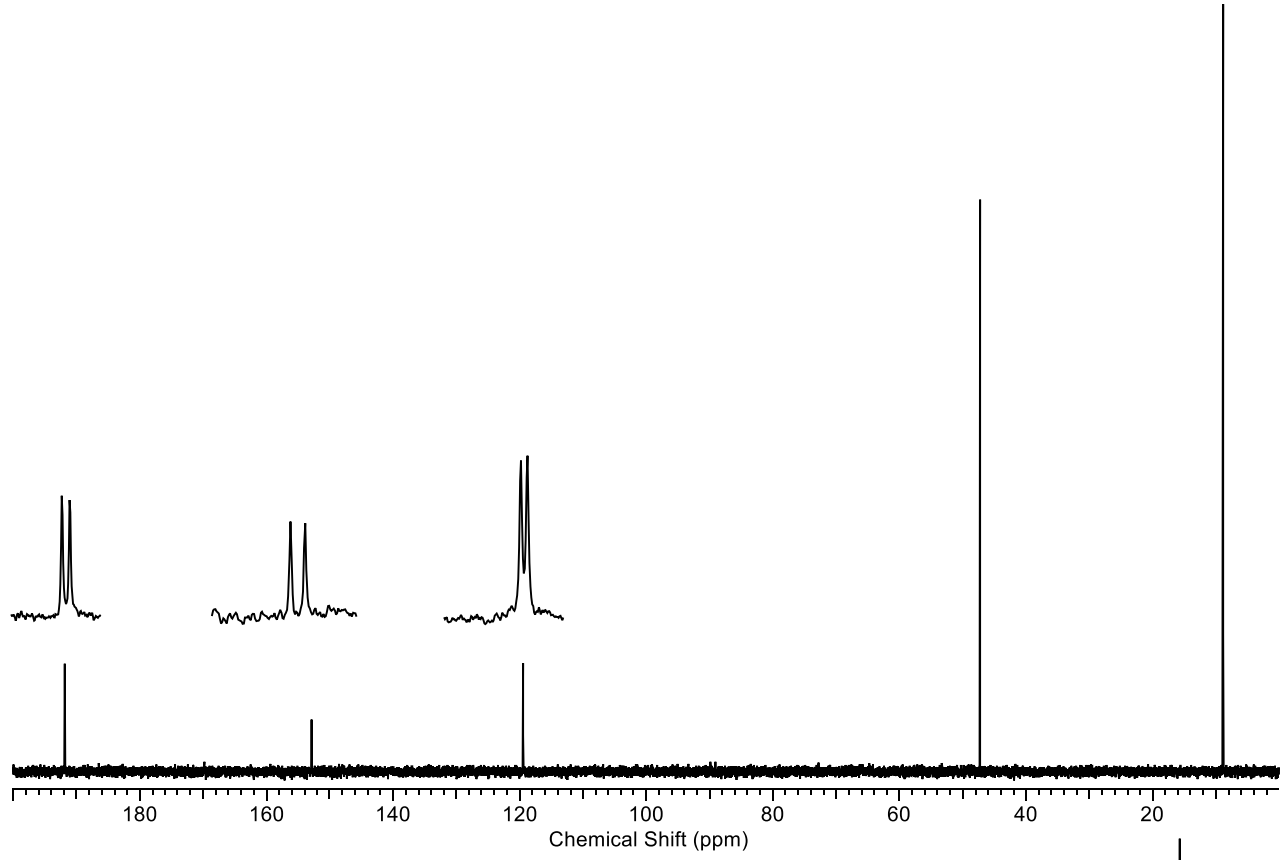


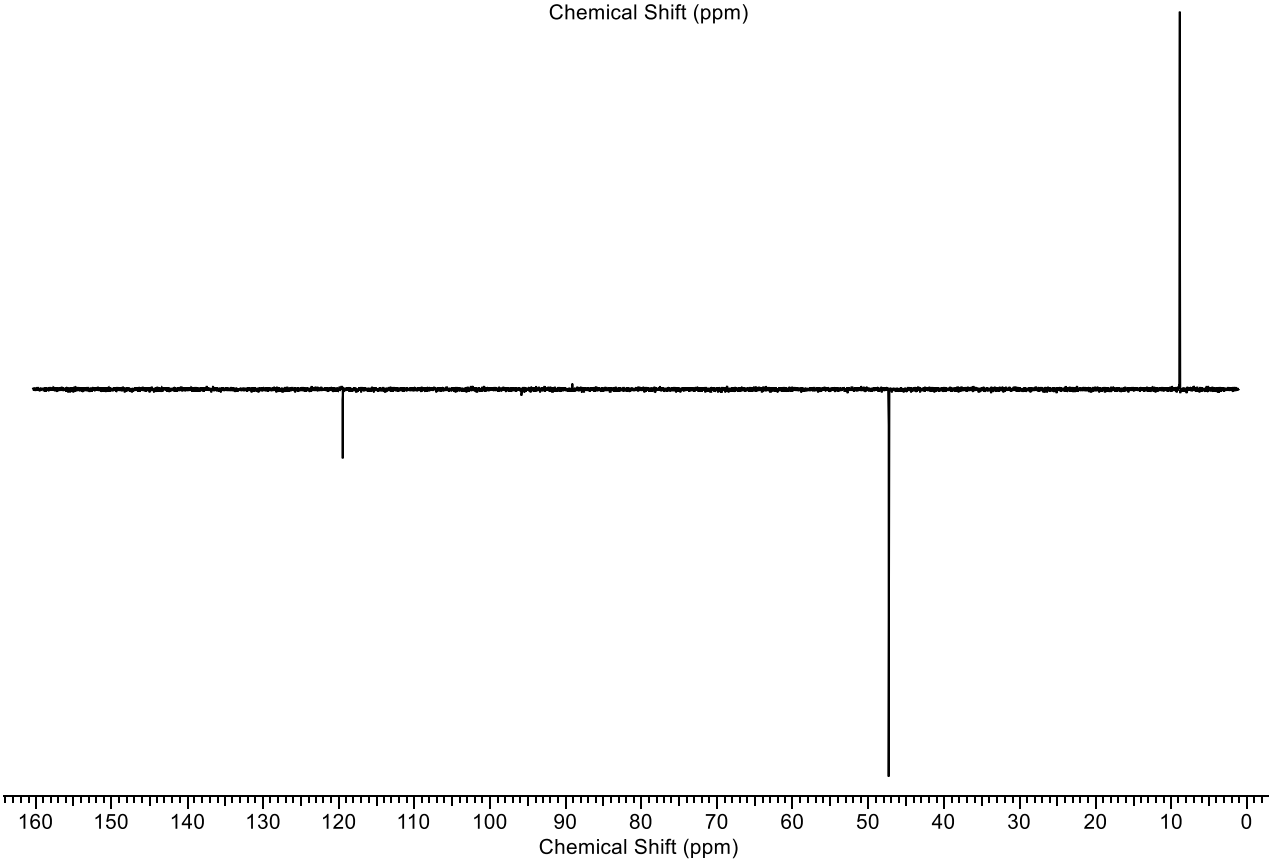
A69: ^{1}H-^{1}H COSY NMR spectrum (600 MHz, {D_2O}) of **105** triethylammonium salt with expansion, showing peaks for the minor, hydrated form, below.



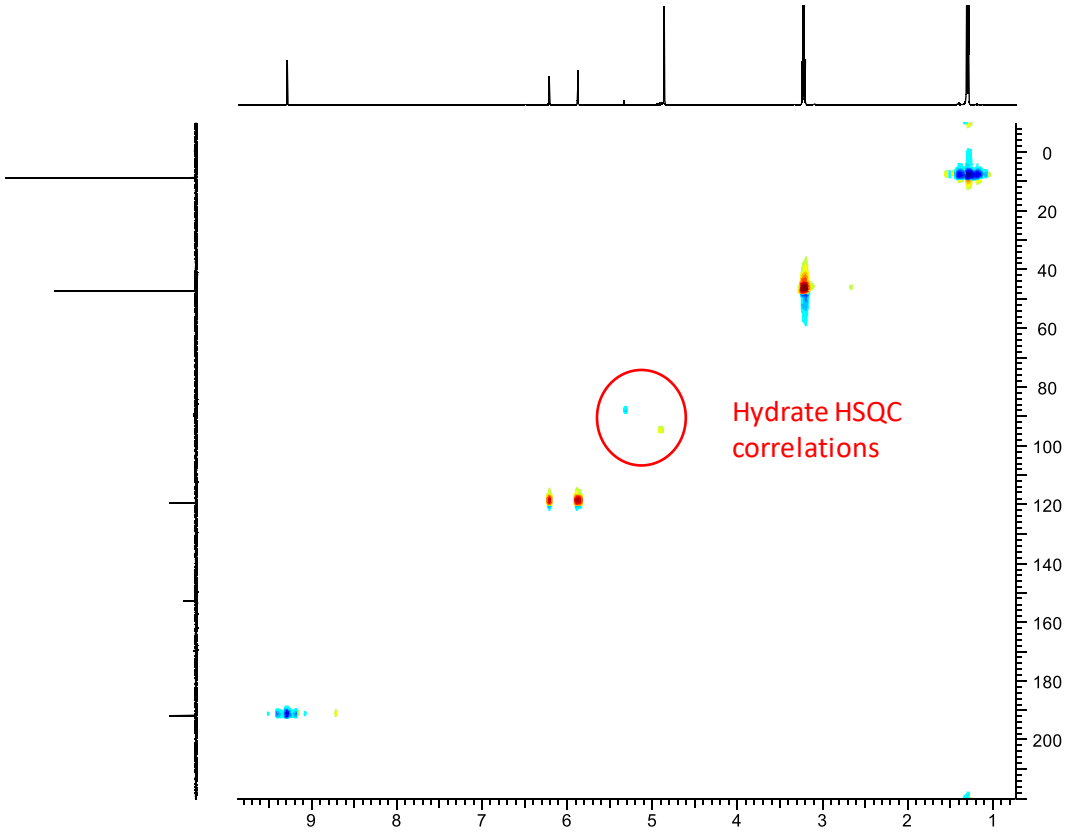

4.60
4.65
4.70
4.75
4.80
4.85
4.90
4.95
5.00
5.05
5.10
5.15
5.20
5.25
5.30
5.35
5.3
5.2
5.1
5.0
4.9
4.8
4.7
4.6

A70: ^{13}C NMR spectrum (151 MHz, {D_2O}, 10 – 200 ppm) **105** triethylammonium salt with expansions overlaid and DEPT 135 spectrum (0 – 165 ppm) below.

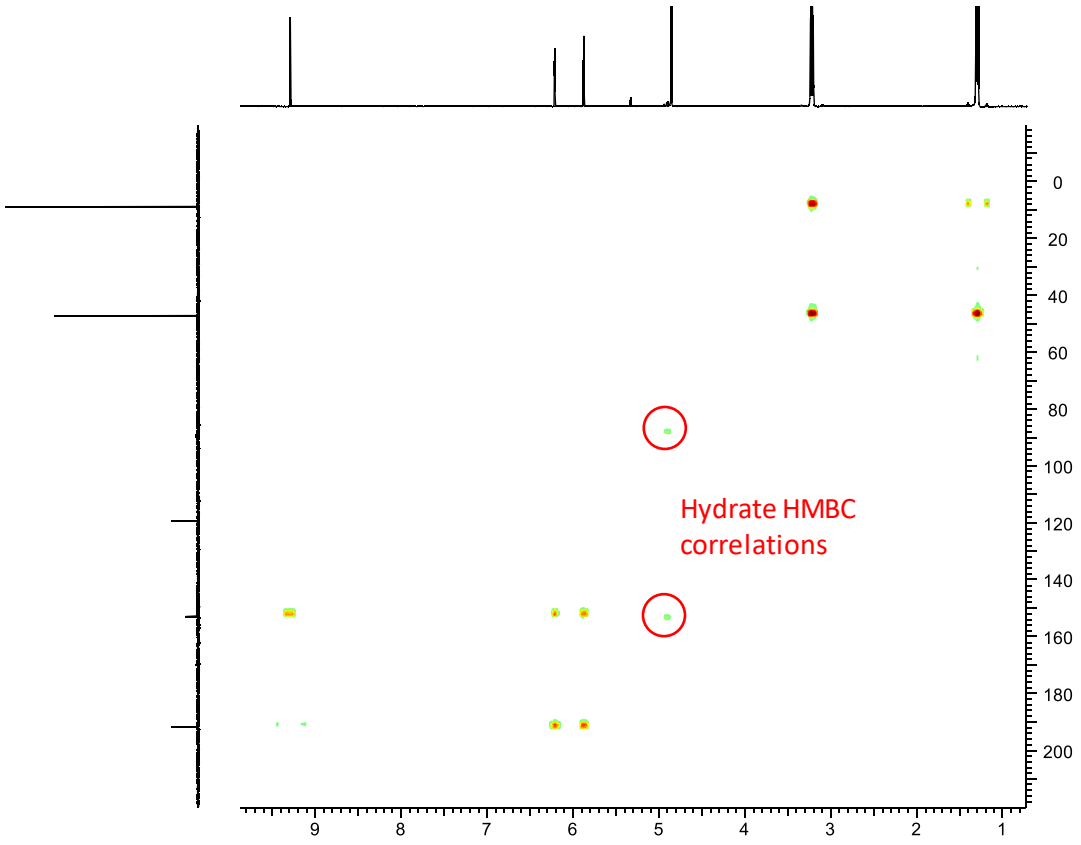




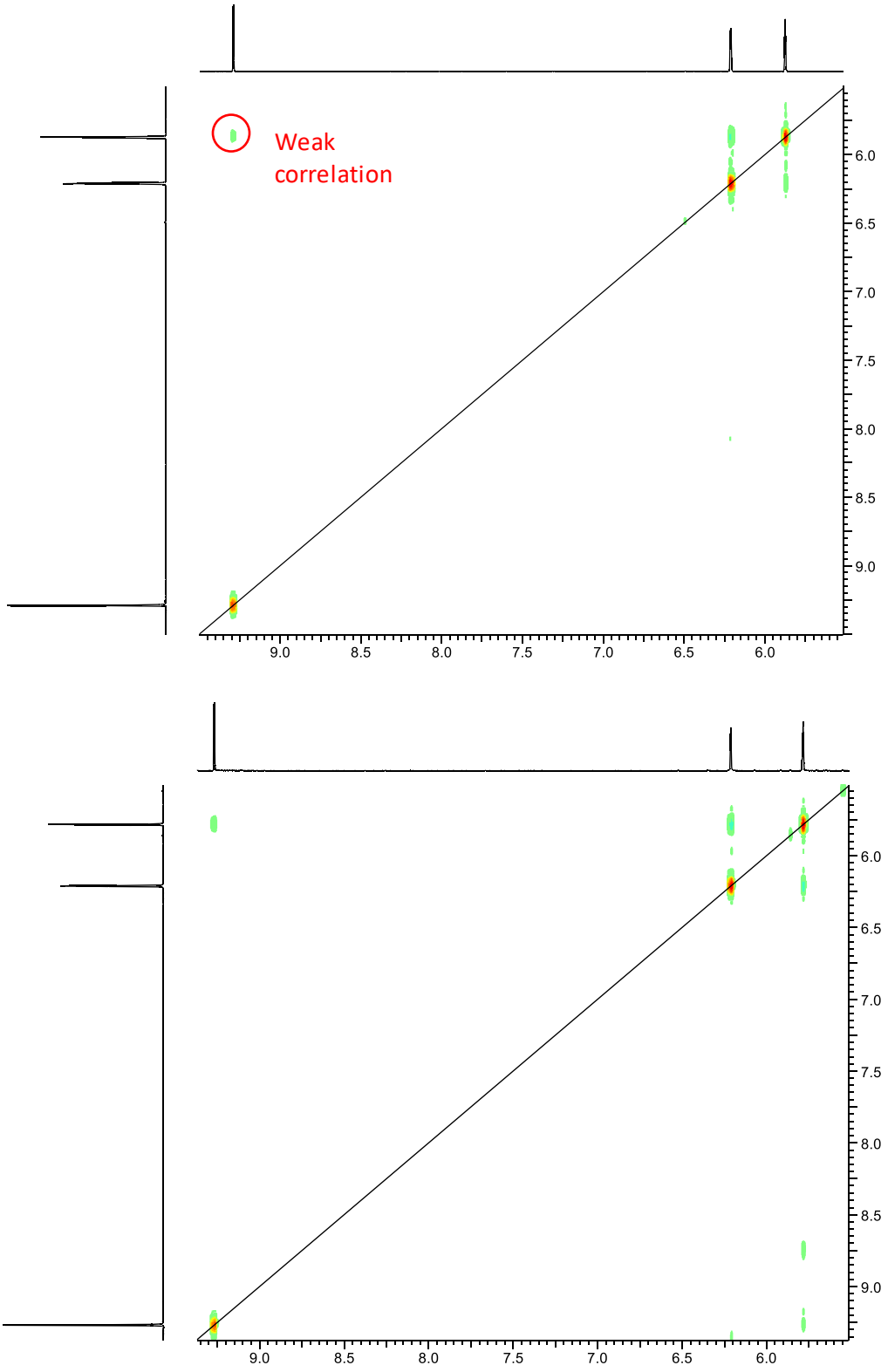
A71: ^{1}H-^{13}C HSQC NMR spectrum (600 MHz, {D_2O}) of **105** triethylammonium salt. The HSQC correlations due to the minor, hydrated form (from which its ^{13}C chemical shifts are inferred) are highlighted.



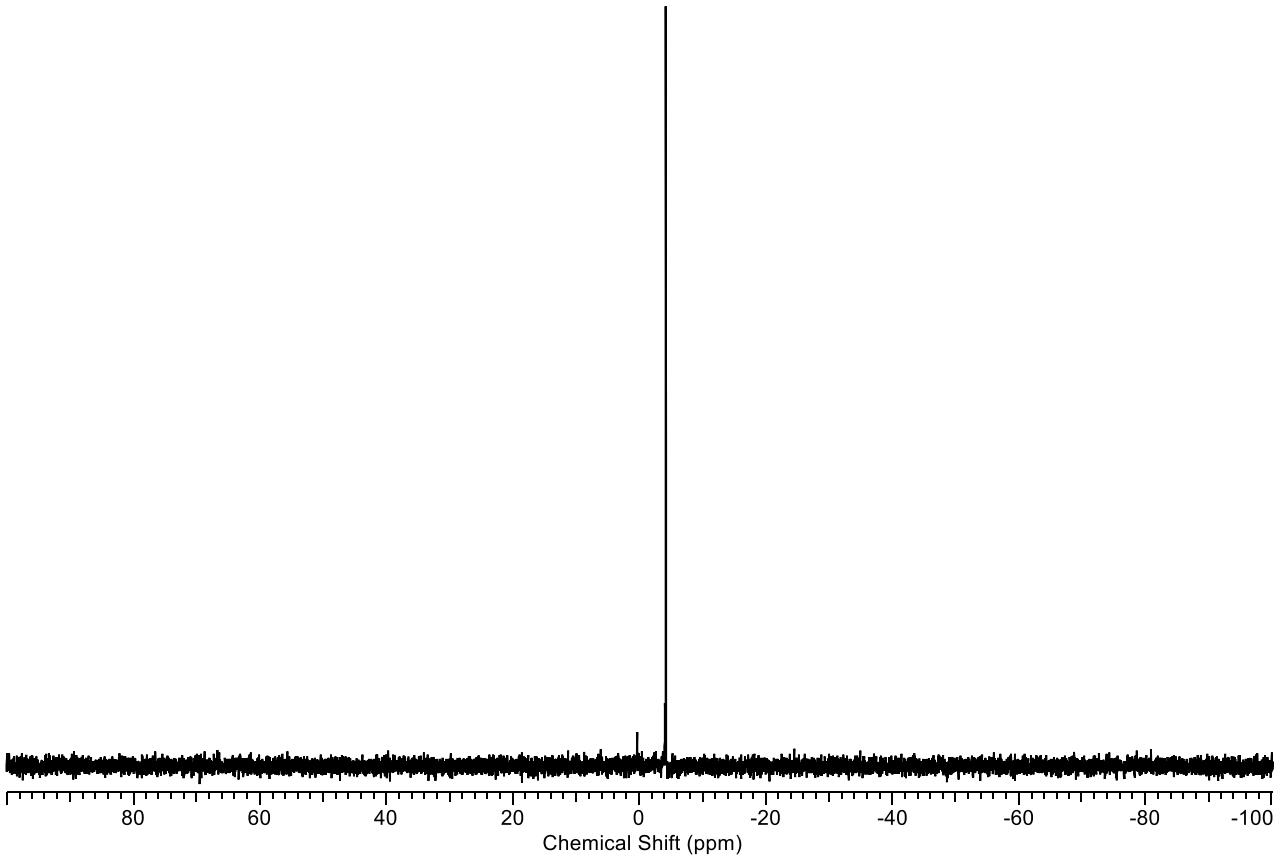
A72: ^{1}H-^{13}C HMBC NMR spectrum (600 MHz, {D_2O}) of **105** triethylammonium salt. The HMBC correlations due to the minor, hydrated form (from which its ^{13}C chemical shifts are inferred) are highlighted.



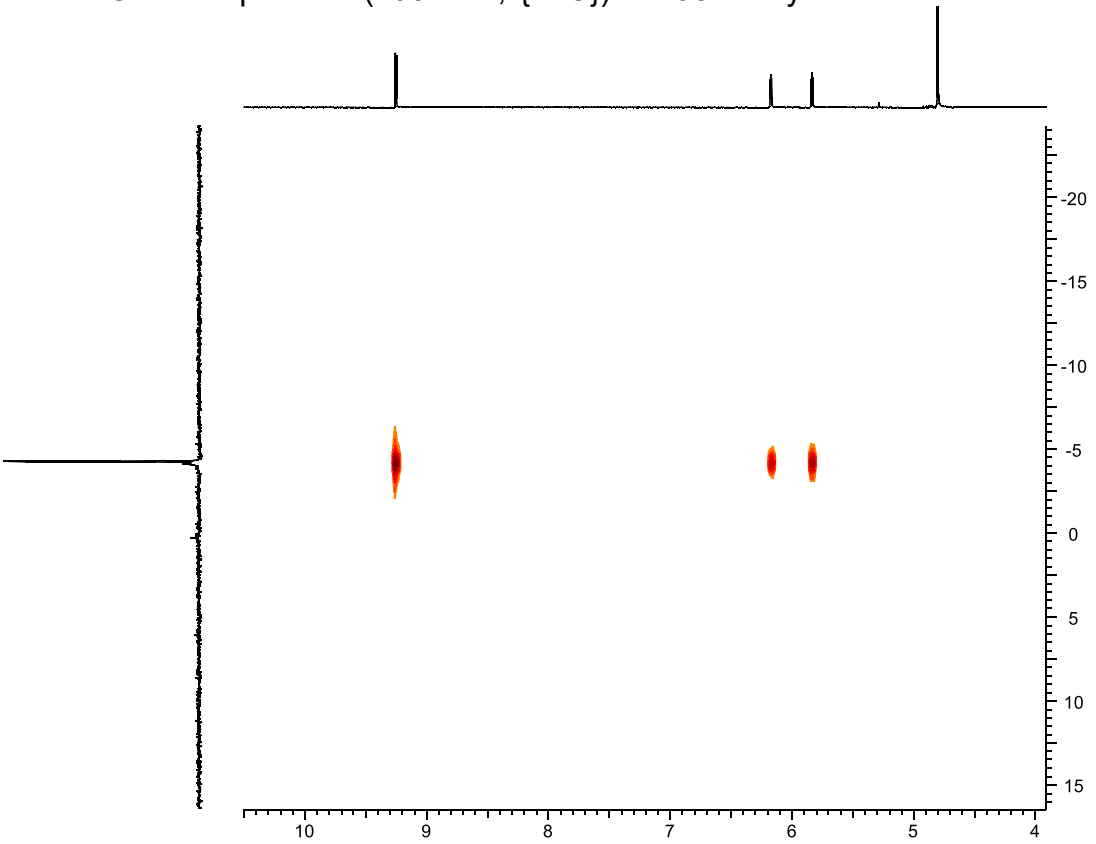
A73: ^{1}H-^{1}H NOESY spectrum (600 MHz, {D_2O}) of **105** triethylammonium salt. A possible weak interaction between one of the geminal protons and the aldehyde proton is highlighted. Shown below is the same experiment carried out on the sodium salt of **105**, displaying the same cross peak (the expected mirror peak is also possibly present here).



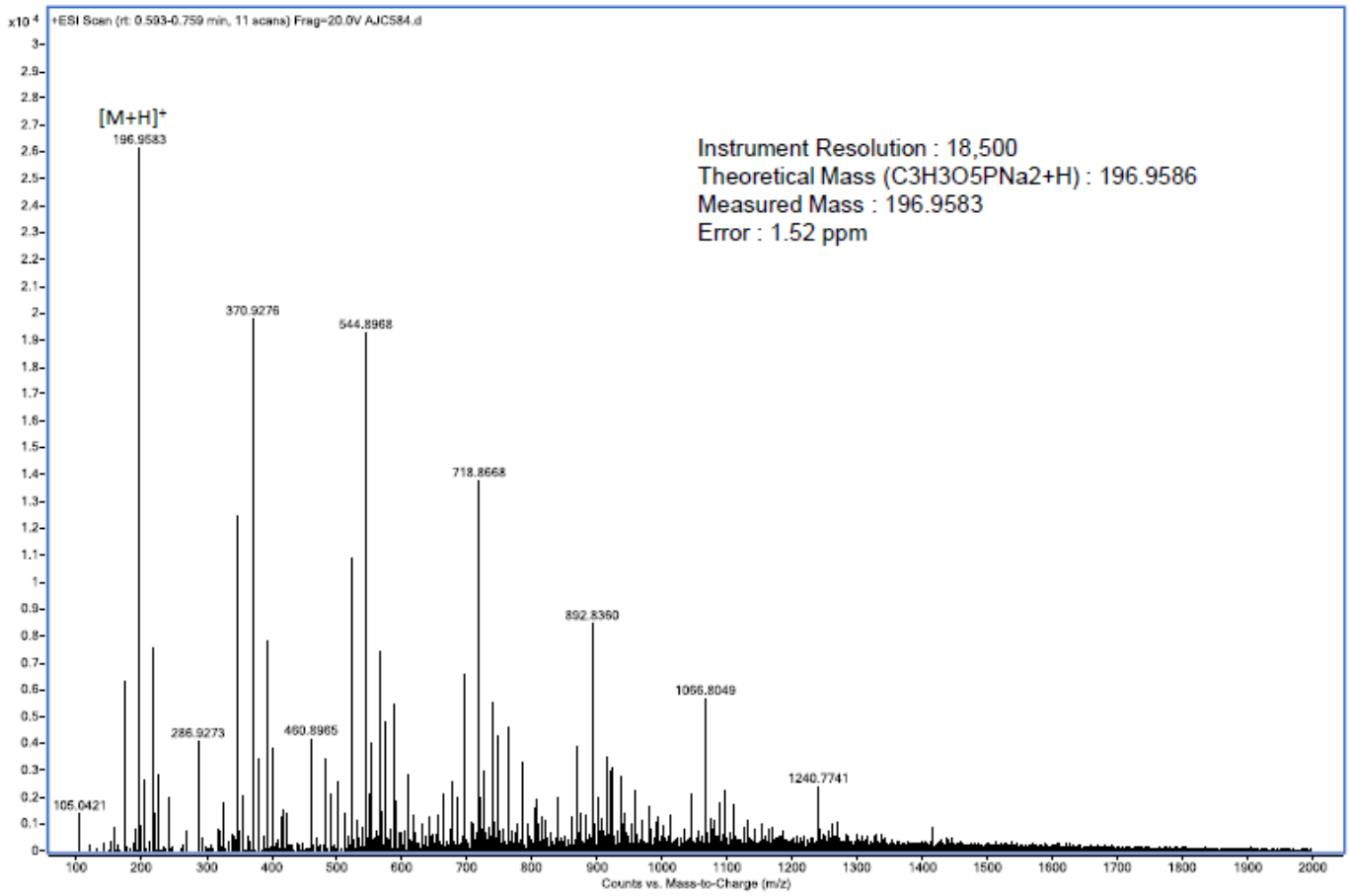
A74: ^{31}P NMR spectrum (161 MHz, {D_2O}, -100 – 100 ppm) of **105** triethylammonium salt.



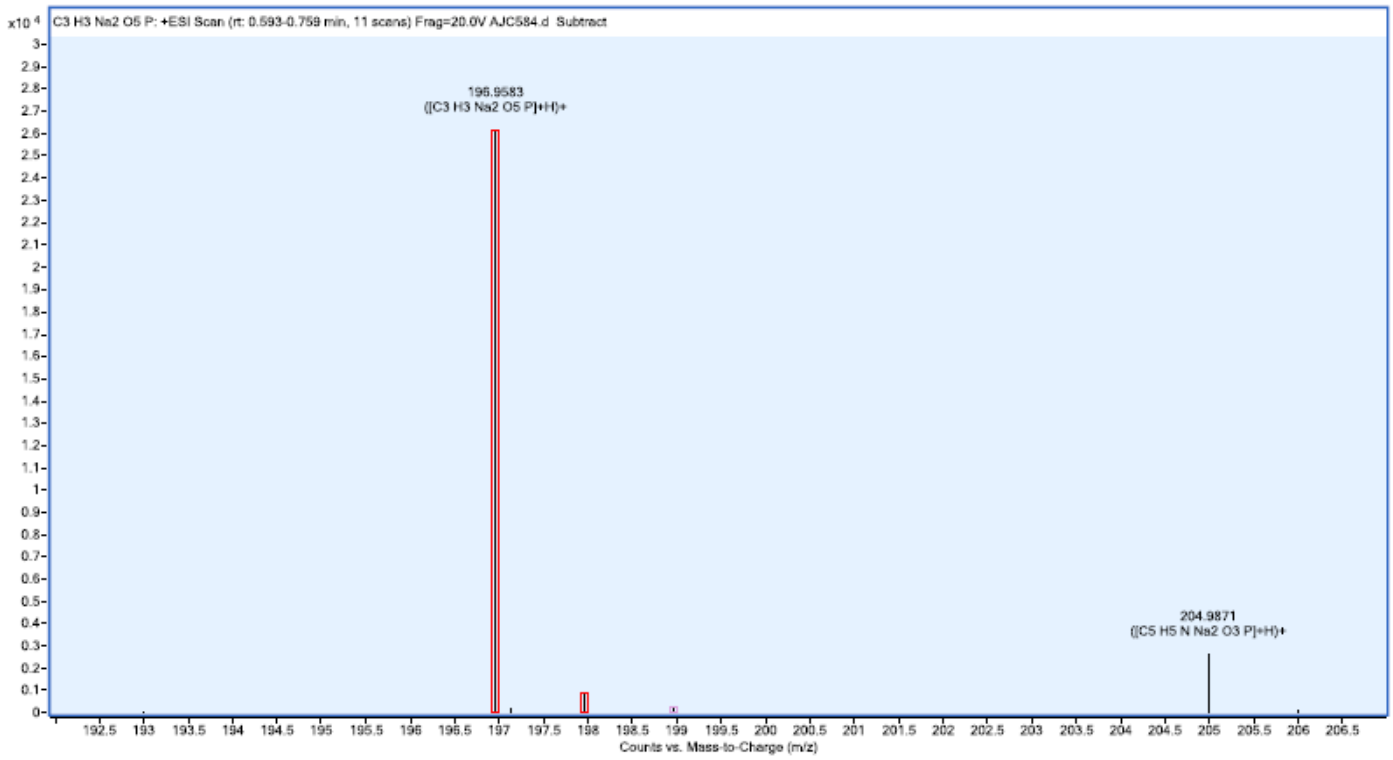
A75: ^{1}H-^{31}P HMBC NMR spectrum (400 MHz, {D_2O}) of **105** triethylammonium salt.



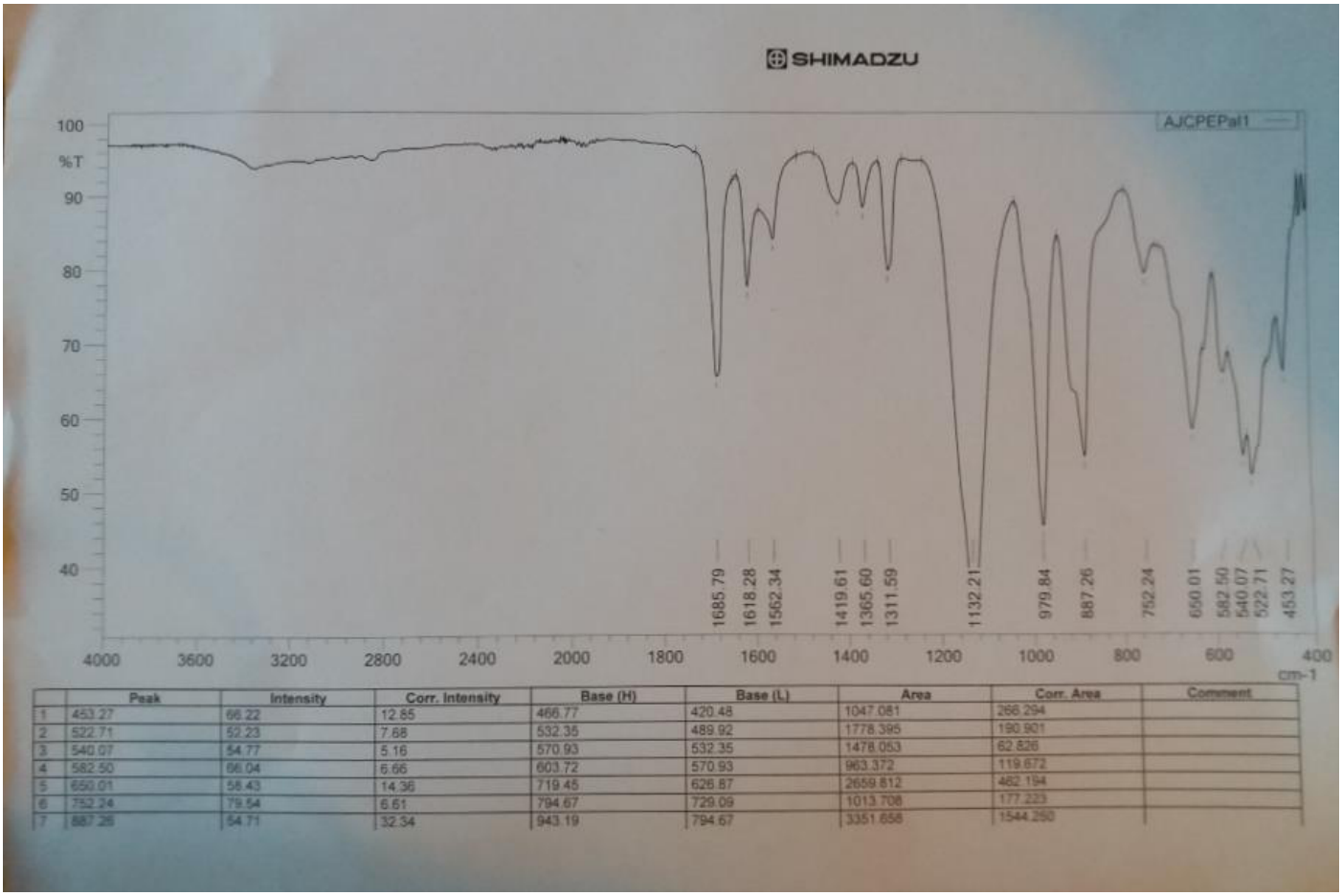
A76: ESI+ mass spectrum of **105**.



A77: HRMS of **105**.



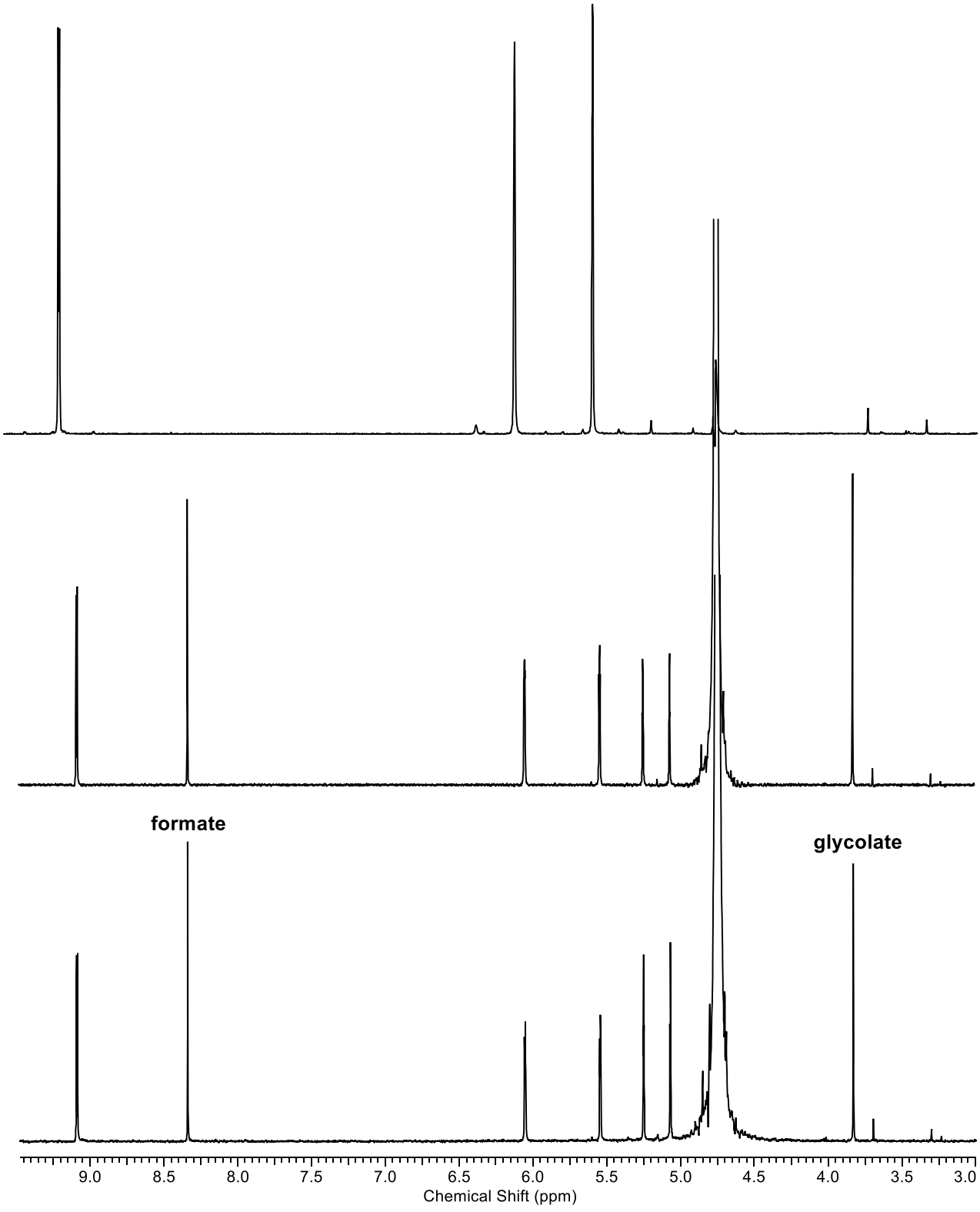
A78: IR spectrum of**105**.



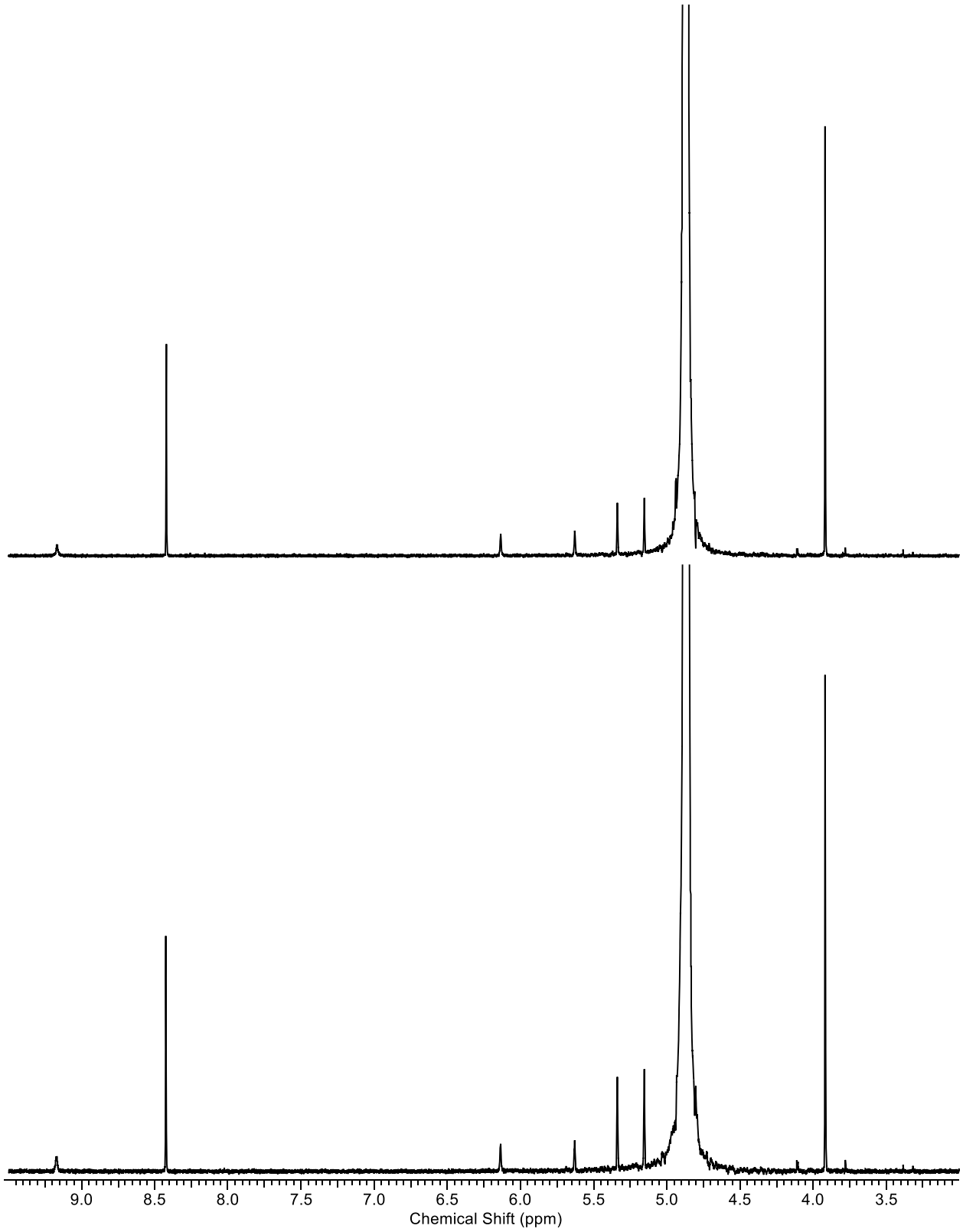
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	453.27	66.22	12.85	466.77	420.48	1047.081	266.294	
2	522.71	52.23	7.68	532.35	489.92	1778.395	190.901	
3	540.07	54.77	5.16	570.93	532.35	1478.053	62.826	
4	582.50	66.04	6.66	603.72	570.93	963.372	119.672	
5	650.01	58.43	14.36	719.45	626.87	2659.812	482.194	
6	752.24	79.54	6.51	794.67	729.09	1013.708	177.223	
7	887.26	54.71	32.34	943.19	794.67	3351.658	1544.250	

Oxidation experiments

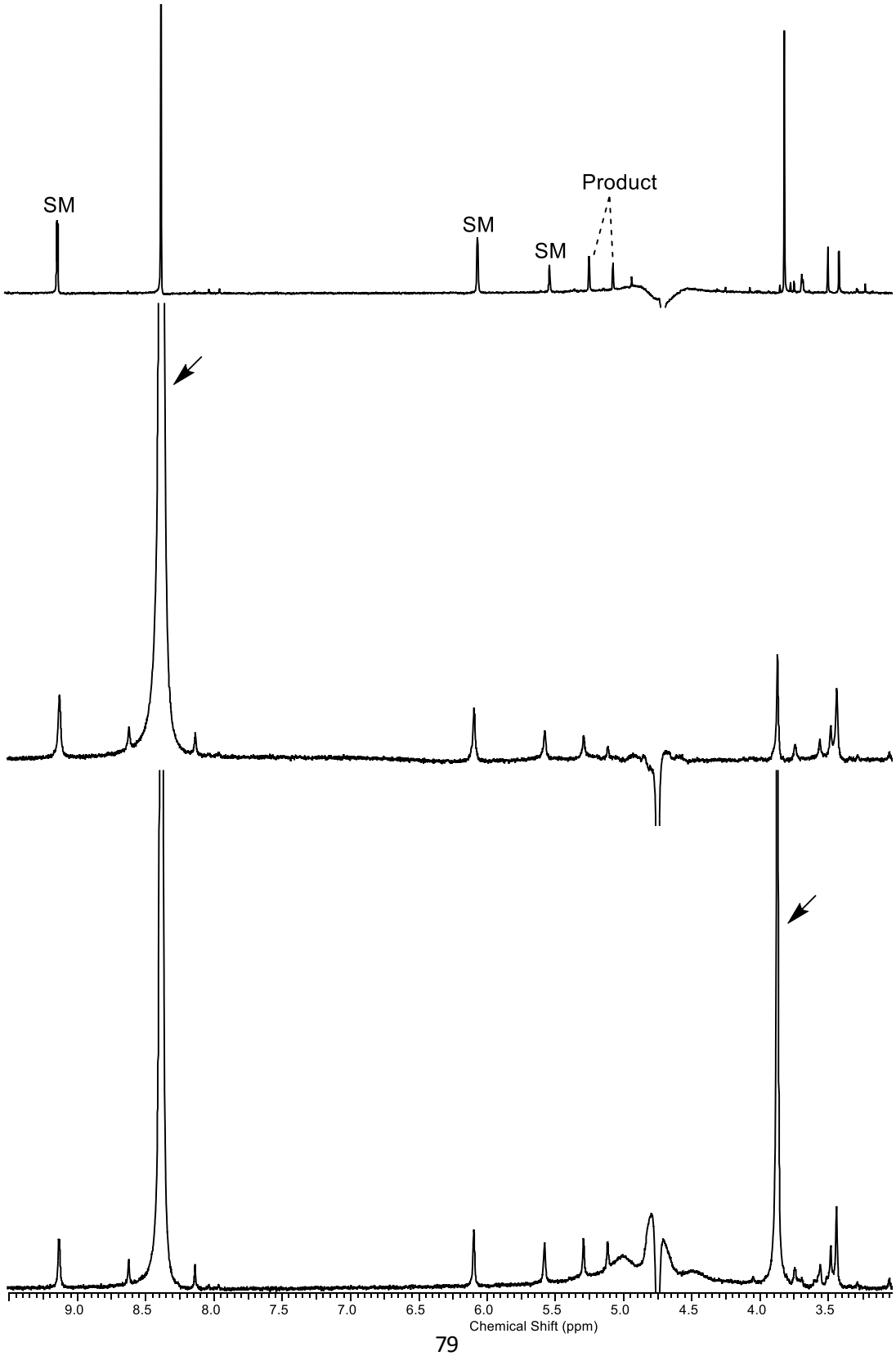
A79: ^{1}H NMR spectra (400 MHz, {D_2O}, 3.0–9.5 ppm) of **Top**, phosphoenolpyruvaldehyde (**105**, 25mM), **Middle**, after incubation with iron (II) chloride (25mM) and hydrogen peroxide (100mM) in phosphate buffer (750mM) at 0 – 25 °C, pH 10, 1 h, followed by lyophilisation and re-dissolution in D_2O and **Bottom**, after spiking with commercial phosphoenol pyruvate (**94**).



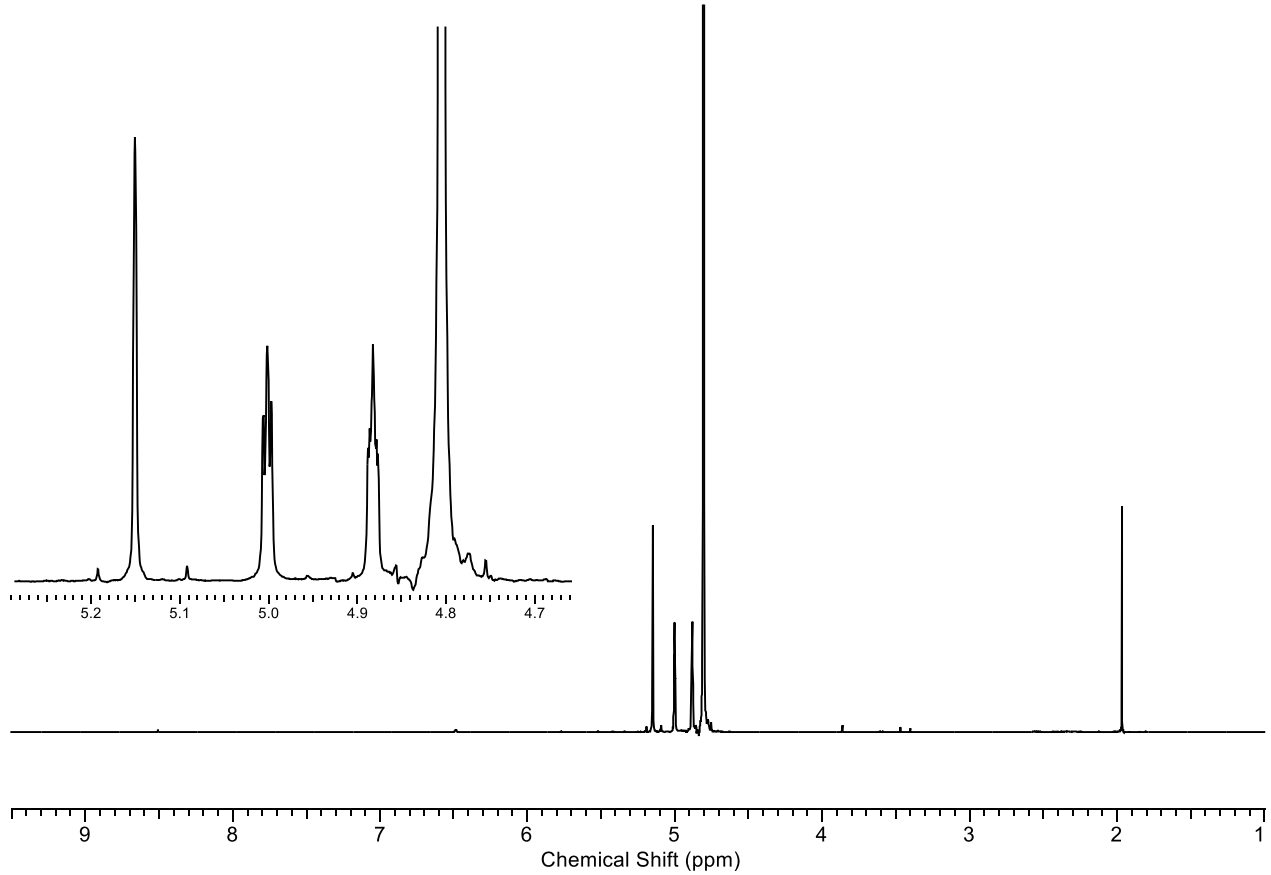
A80: ^{1}H NMR spectra (400 MHz, {D_2O}, 3.0–9.5 ppm) of **Top**, phosphoenolpyruvaldehyde (**105**, 25mM) after incubation with hydrogen peroxide (50mM) in phosphate buffer (750mM) at 0 – 25 °C, pH 10, 1 h, followed by evaporation and re-dissolution in D_2O and **Bottom**, after spiking with commercial phosphoenol pyruvate (**94**).



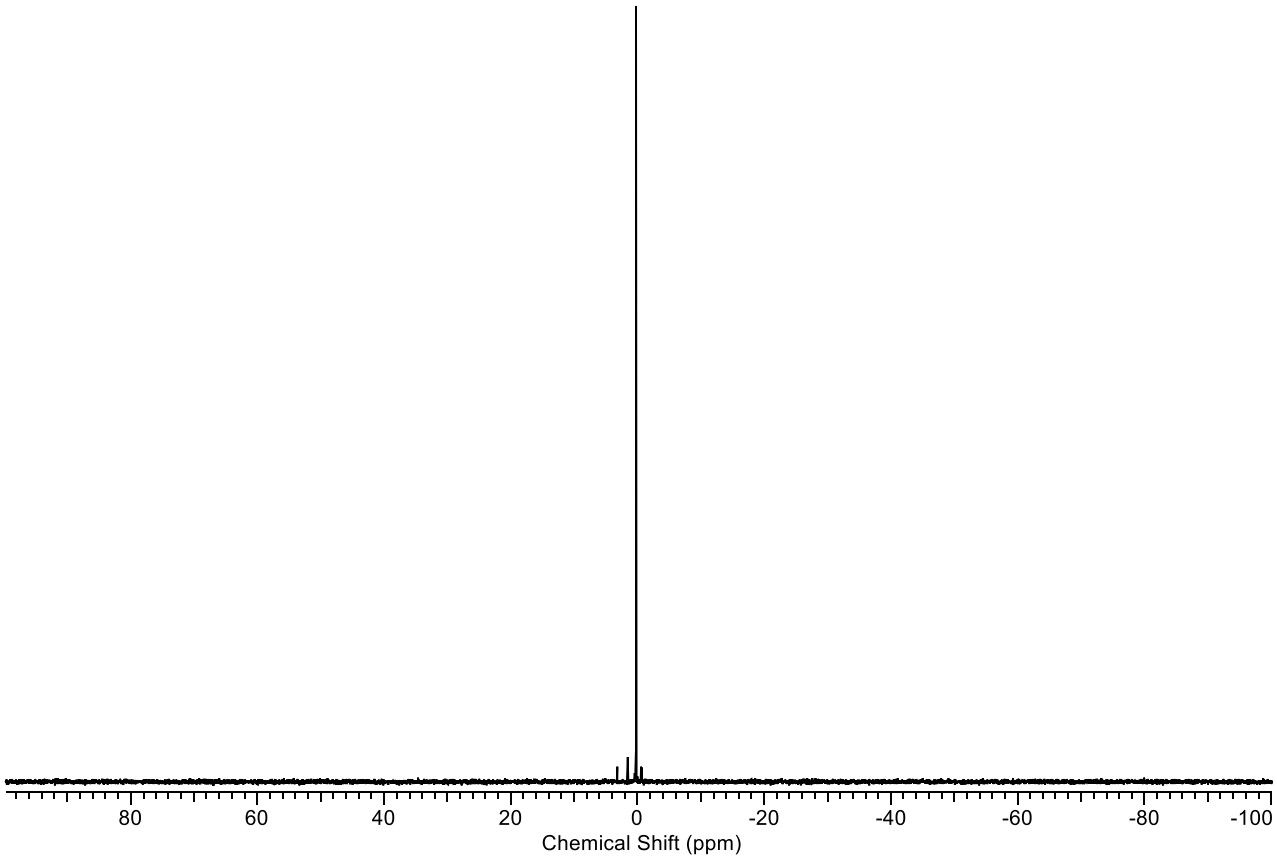
A81: ^{1}H NMR spectra (400 MHz, {H_2O/D_2O, 9:1}, 3.0–9.5 ppm) of **Top**, the reaction of phosphoenolpyruvaldehyde (**105**, 25mM) with iron (II) chloride (25mM) and hydrogen peroxide (400mM) in water at 0 – 25 °C, contact pH (final pH = 8.1), 2 h, after shaking with Chelex™ resin and filtering; **Middle**, after spiking with commercial formic acid and **Bottom**, after spiking with commercial glycolic acid. Amplified peaks are indicated.



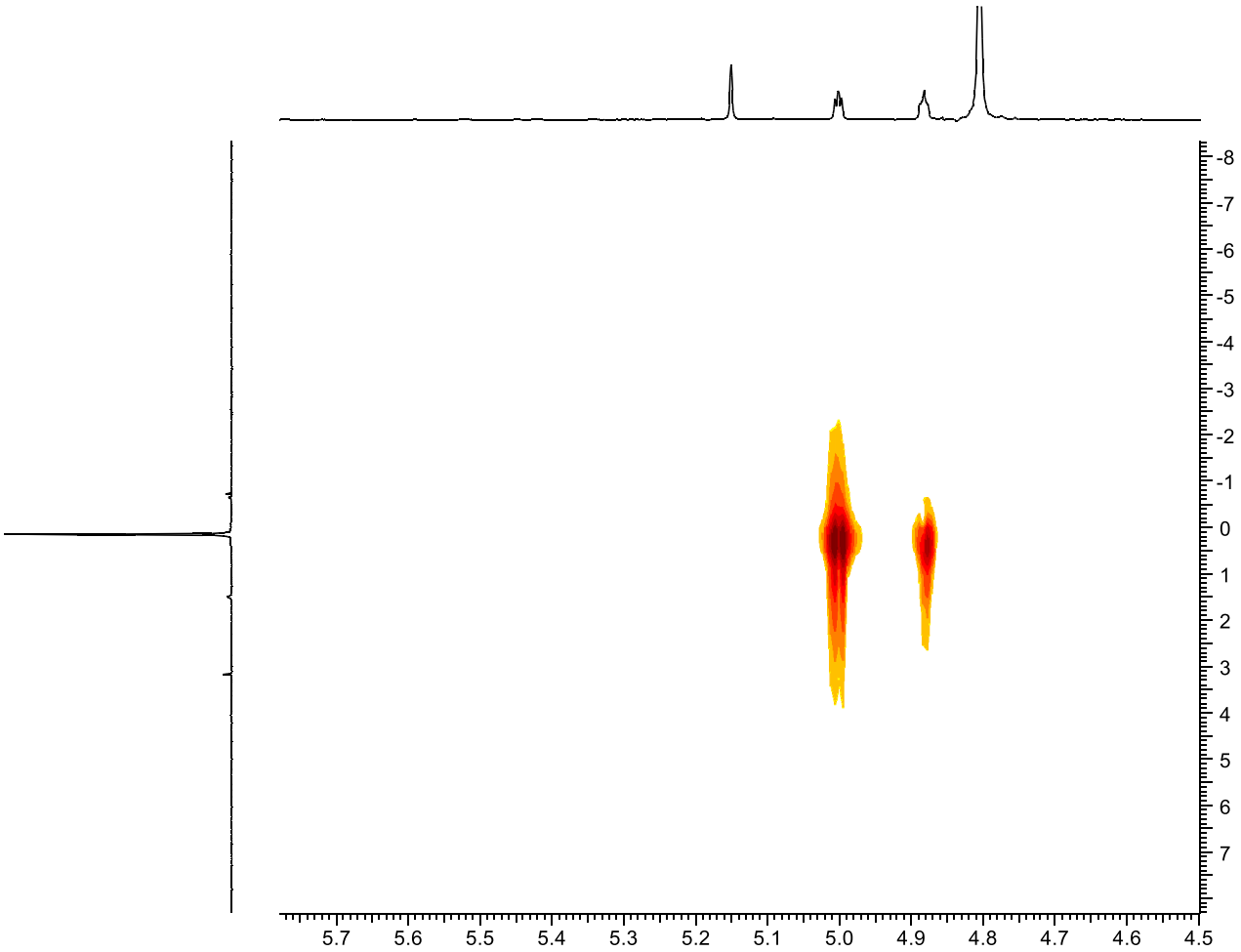
A82: ^{1}H NMR spectrum (600 MHz, {D_2O}, 1.0 – 9.5 ppm) of phosphoenolpyruvaldehyde cyanohydrin (**119**) formed on incubation of phosphoenolpyruvaldehyde (**105**, 200mM) with sodium cyanide (1M) and acetic acid at ambient temperature, pD 9.5, 2 h, with expansion (4.7 – 5.3 ppm) overlaid.



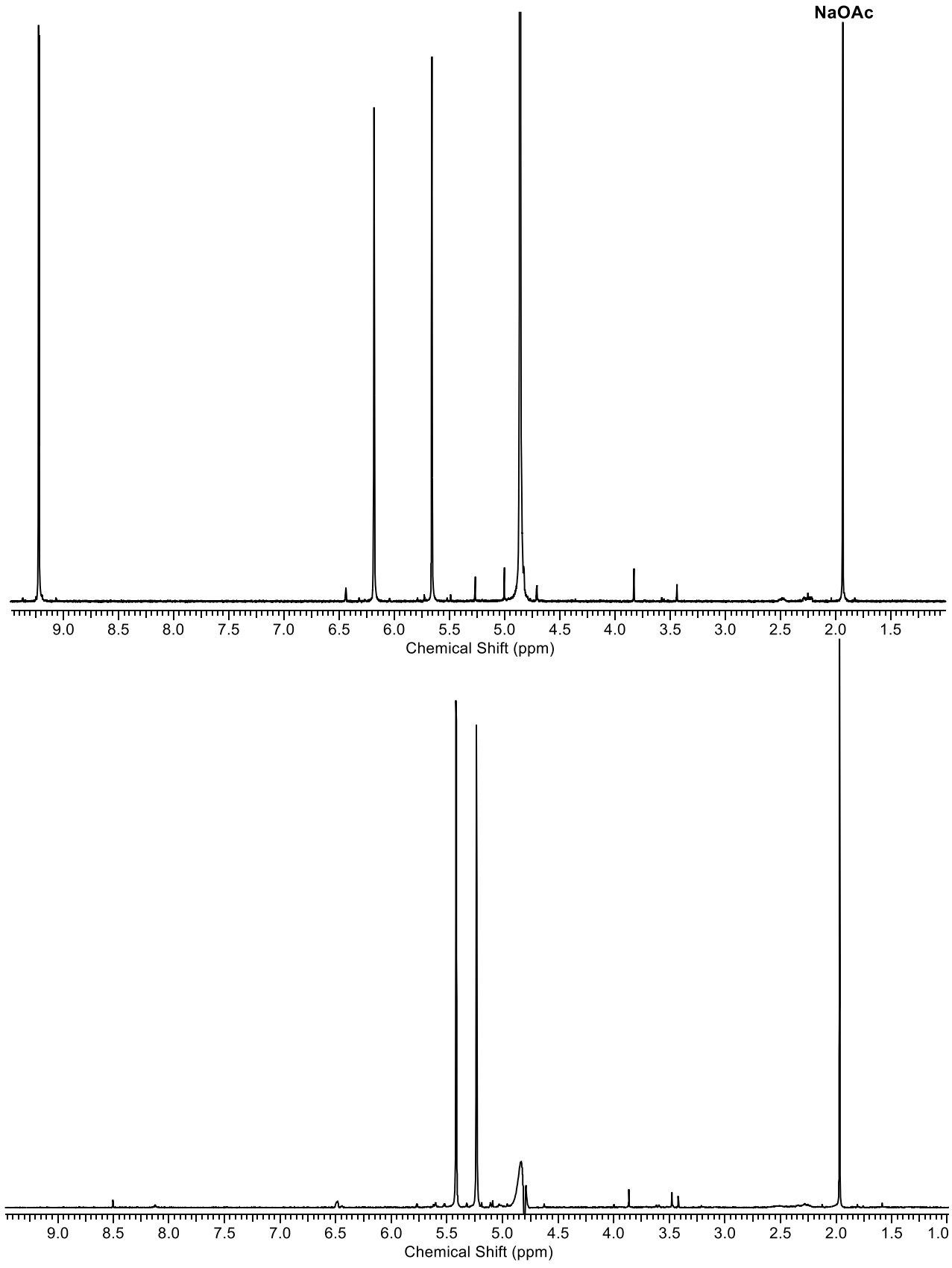
A83: 31P NMR spectrum (161 MHz, {D_2O}, -100 – 100 ppm) of phosphoenolpyruvaldehyde cyanohydrin (**119**) formed on incubation of phosphoenolpyruvaldehyde (**105**, 200mM) with sodium cyanide (1M) and acetic acid at ambient temperature, pD 9.5, 2 h.



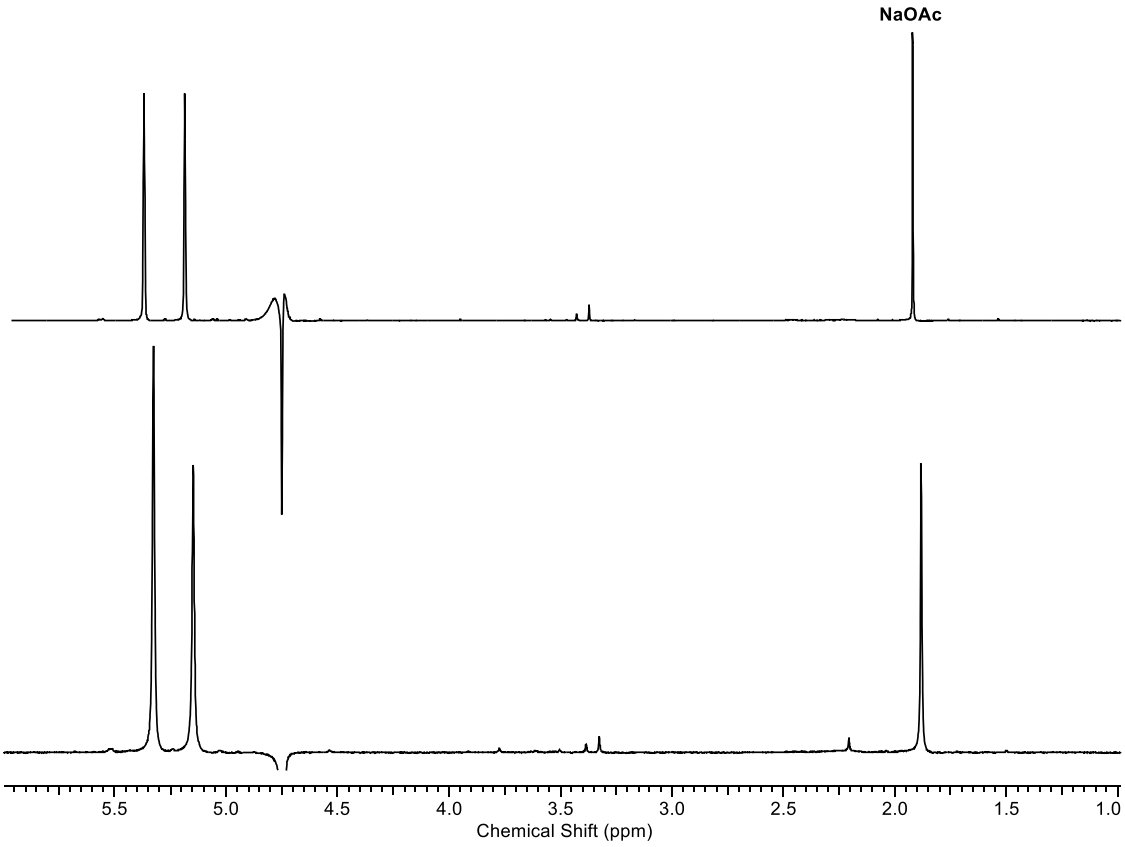
A84: ^{1}H-^{31}P HMBC NMR spectrum (400 MHz, {D_2O}) of phosphoenolpyruvaldehyde cyanohydrin (**119**) formed on incubation of phosphoenolpyruvaldehyde (**105**, 200mM) with sodium cyanide (1M) and acetic acid at ambient temperature, pD 9.5, 2 h.



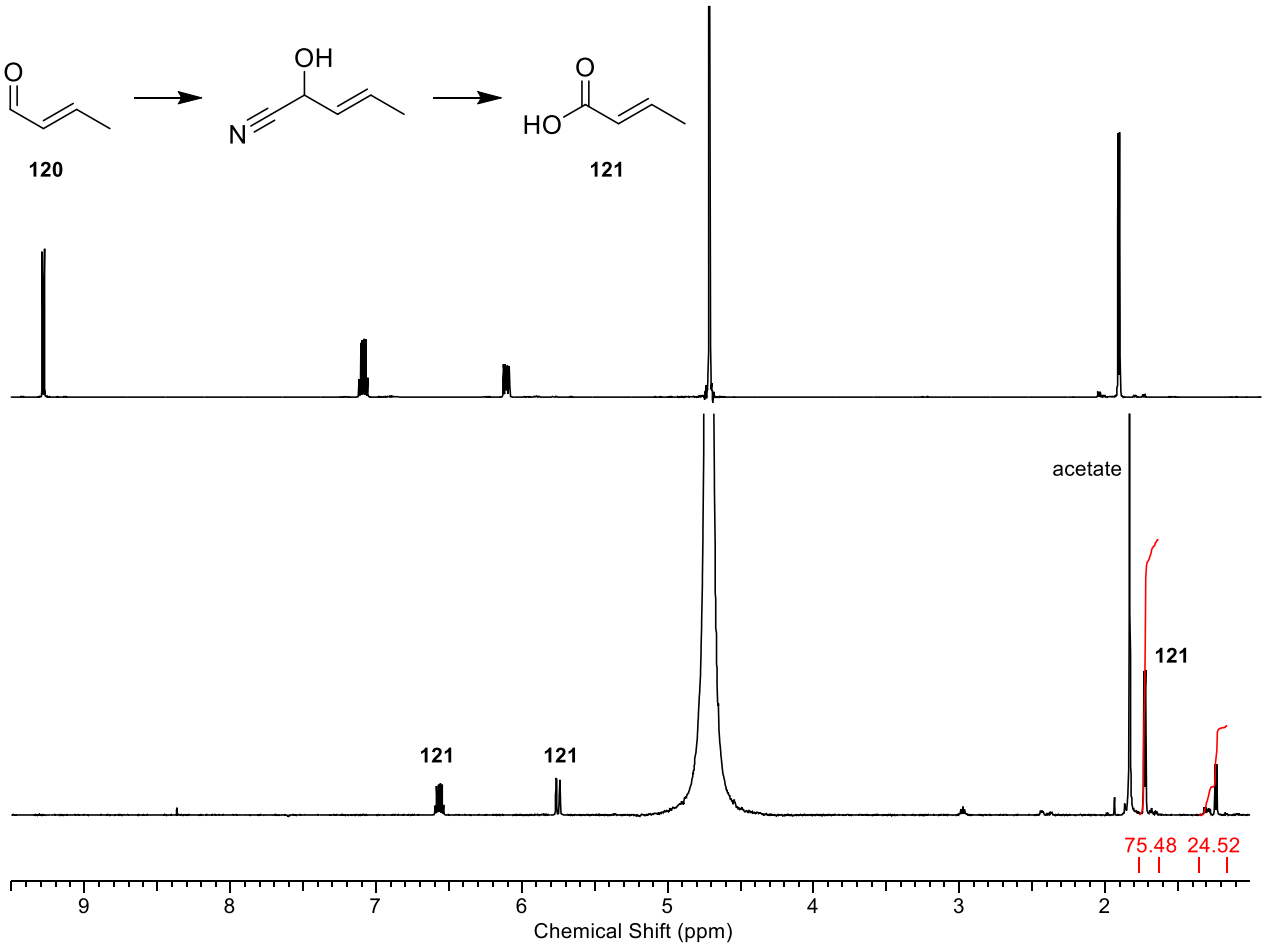
A85: ^{1}H NMR spectra (400 MHz, {H_2O/D_2O, 9:1}, 1.0–9.5 ppm) of **Top**, phosphoenolpyruvaldehyde (**105**, 100mM) with with sodium acetate and **Bottom**, after incubation with sodium cyanide (500mM) and manganese dioxide (20 eq.) at ambient temperature, pH 10.5, 2h.



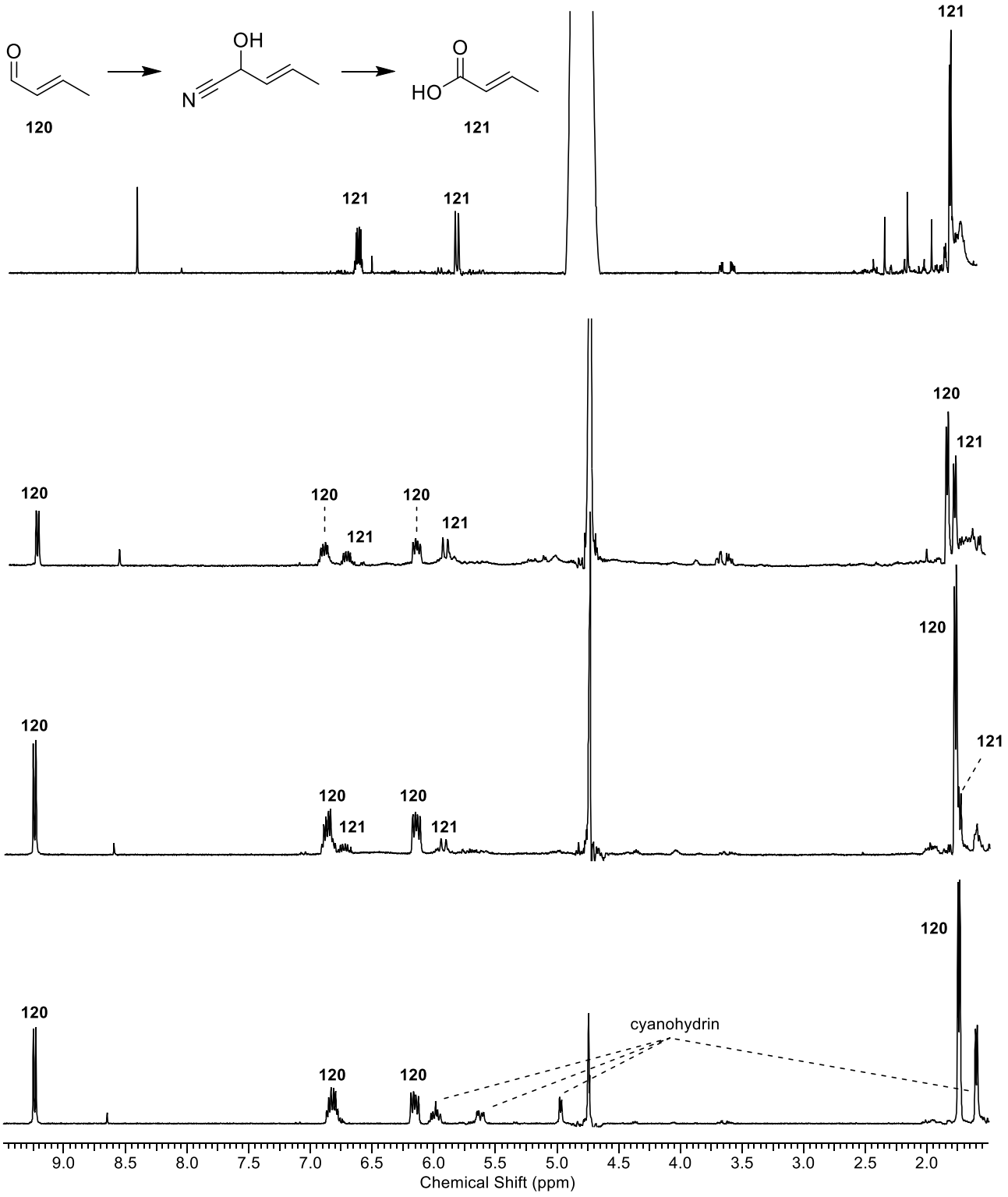
A86: ^{1}H NMR spectra (400 MHz, {H_2O/D_2O, 9:1}, 1.0–6.0 ppm) of **Top**, phosphoenolpyruvaldehyde (**105**, 100mM) with with sodium acetate after incubation with sodium cyanide (500mM) and manganese dioxide (20 eq.) at ambient temperature, pH 9.5, 2h and **Bottom**, after spiking with commercial phosphoenol pyruvate, **94**.



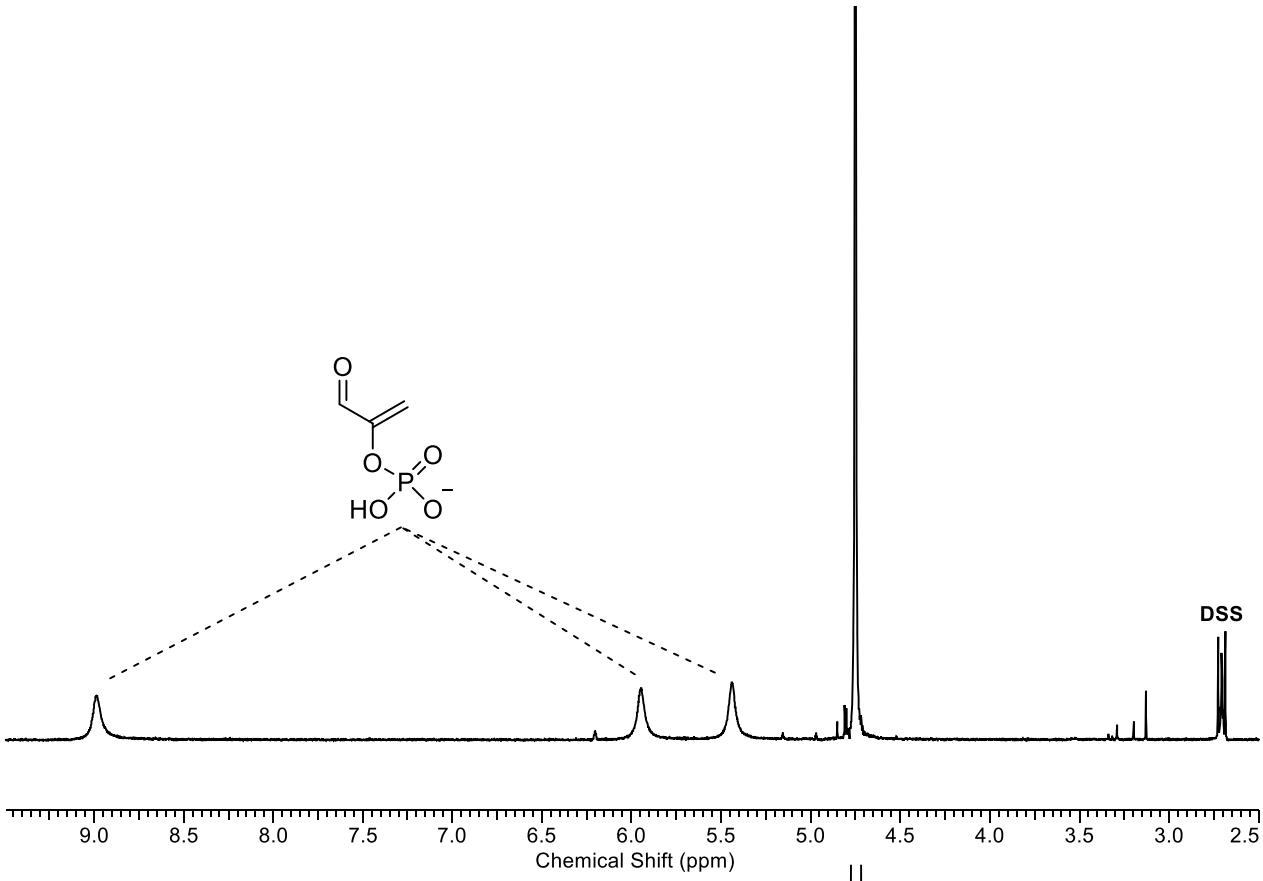
A87: ^{1}H NMR spectra (600 MHz, {D_2O}, 1.0–9.5 ppm) of **Top**, *trans*-crotonaldehyde (**120**) the reaction of *trans*-crotonaldehyde (**120**, 100mM) and **Bottom**, after incubation with sodium cyanide (500mM) and manganese dioxide (20 eq.) and acetic acid at ambient temperature, pD 10, for 2 h.

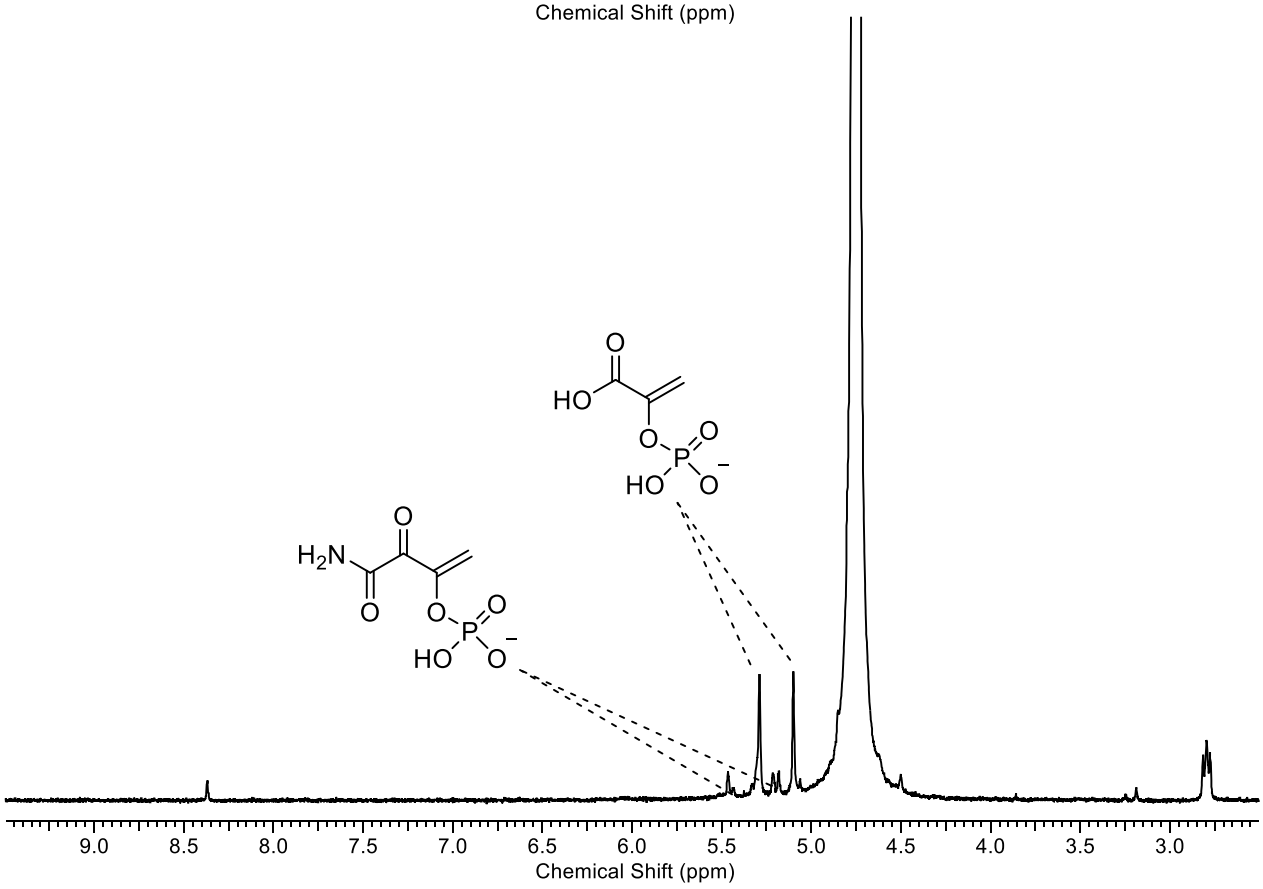


A88: ^{1}H NMR spectra (600 MHz, {1M phosphate, H_2O/D_2O, 9:1}, 1.5–9.5 ppm) of the reaction of *trans*-crotonaldehyde (**120**, 100mM) with sodium cyanide (500mM) and potassium ferricyanide (1M) at ambient temperature, 20 h. In descending order: pH 10.5, pH 9, pH 8 and pH 7.

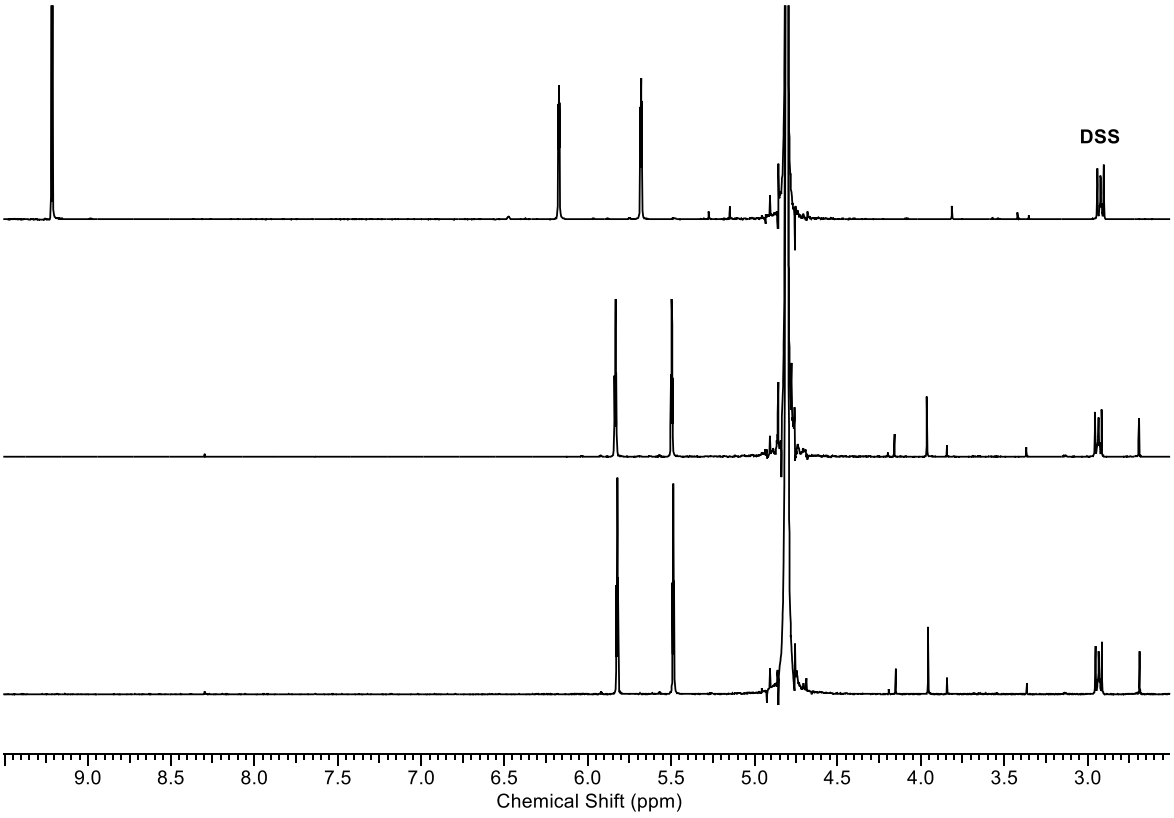


A89: ^{1}H NMR spectra (400 MHz, {750mM phosphate, D_2O}, 2.5–9.5 ppm) of **Top**, phosphoenolpyruvaldehyde (**105**, 33mM) at pD 13 and **Bottom**, after incubation with sodium cyanide (167mM) and potassium ferricyanide (333mM) at ambient temperature, 2h.

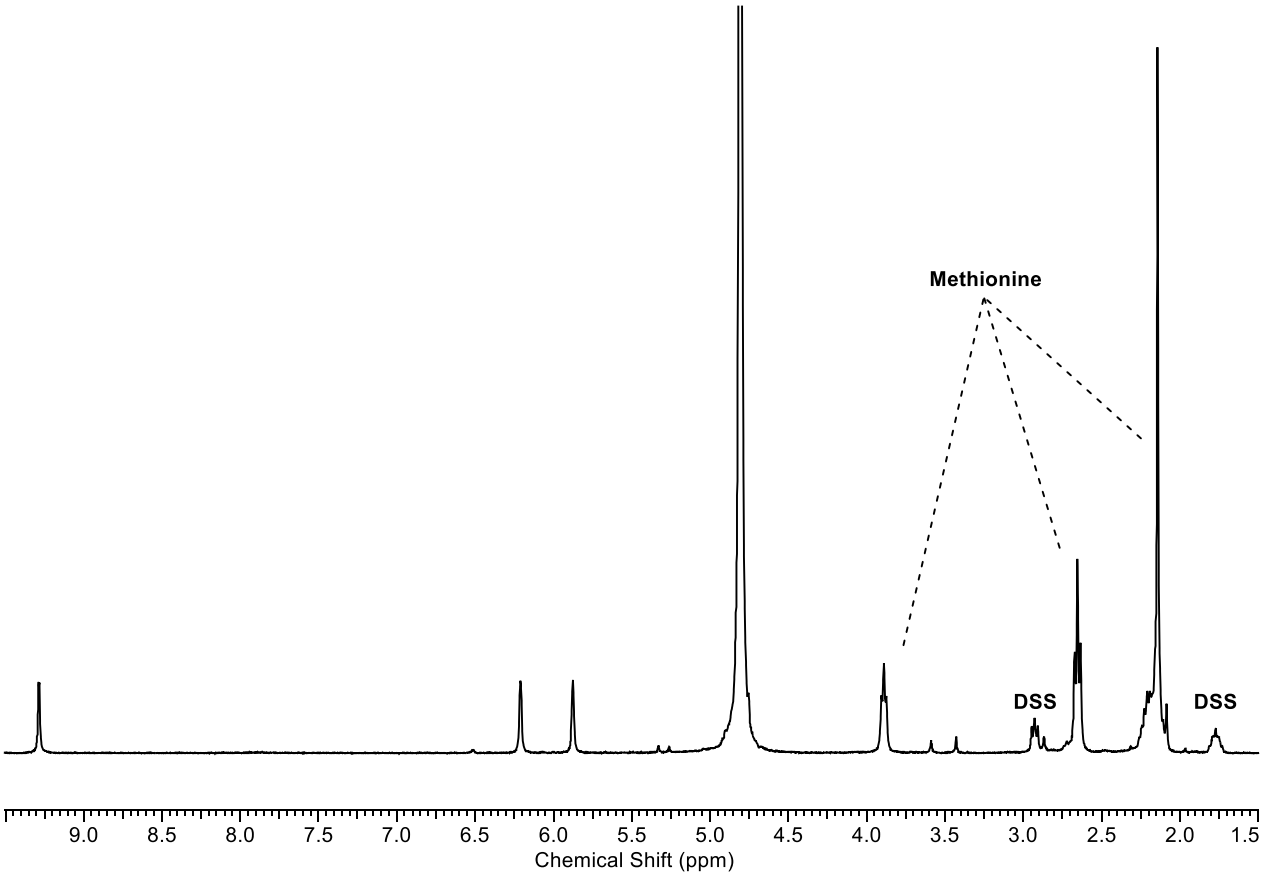


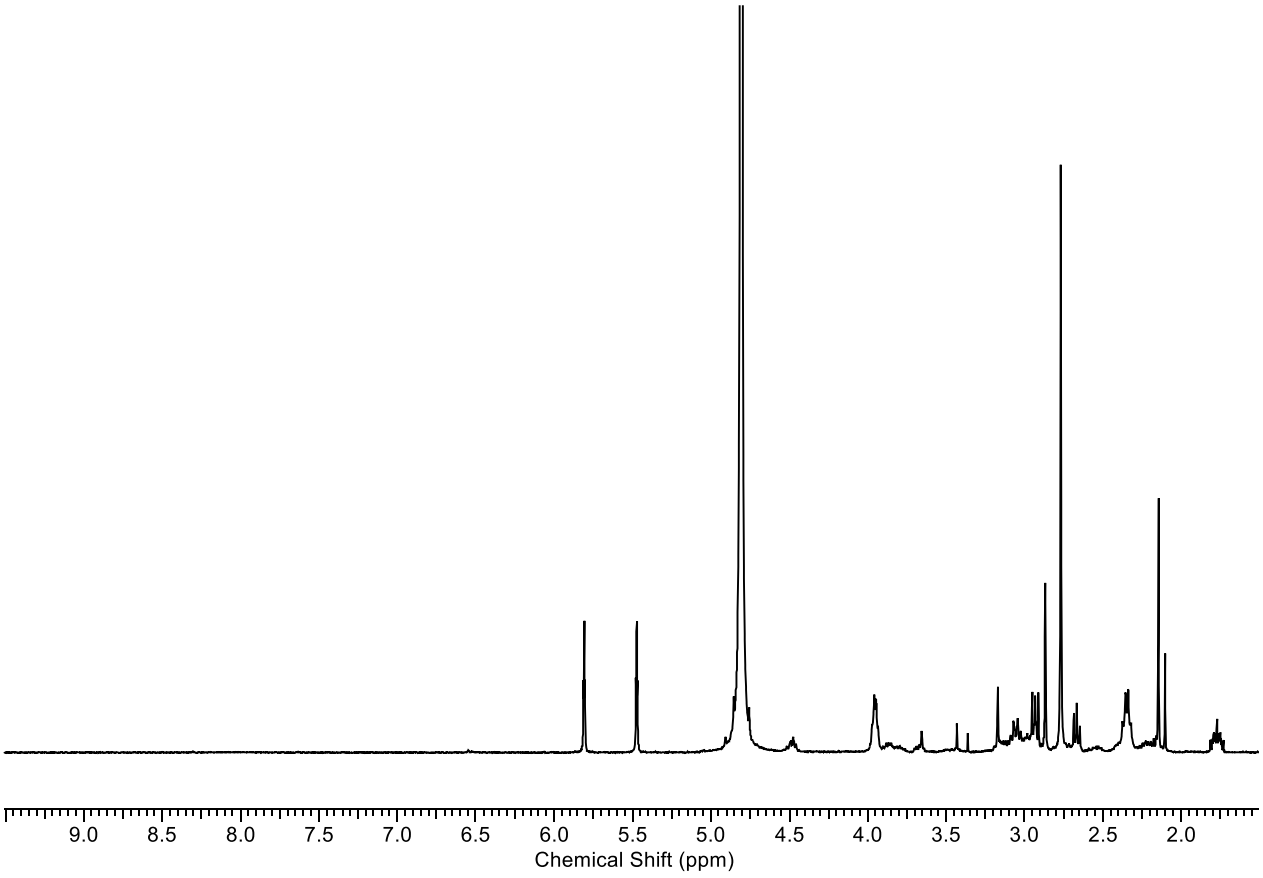


A90: ^{1}H NMR spectra (400 MHz, {60mM phosphate, D_2O}, 2.5–9.5 ppm) of **Top**, phosphoenolpyruvaldehyde (**105**, 70mM) with DSS at pD 4, **Middle**, after incubation with DMSO-d6 (140mM) and sodium chlorite (100mM), 0 °C - ambient temperature, for 2h and **Bottom**, after spiking with commercial phosphoenol pyruvate, **94**.

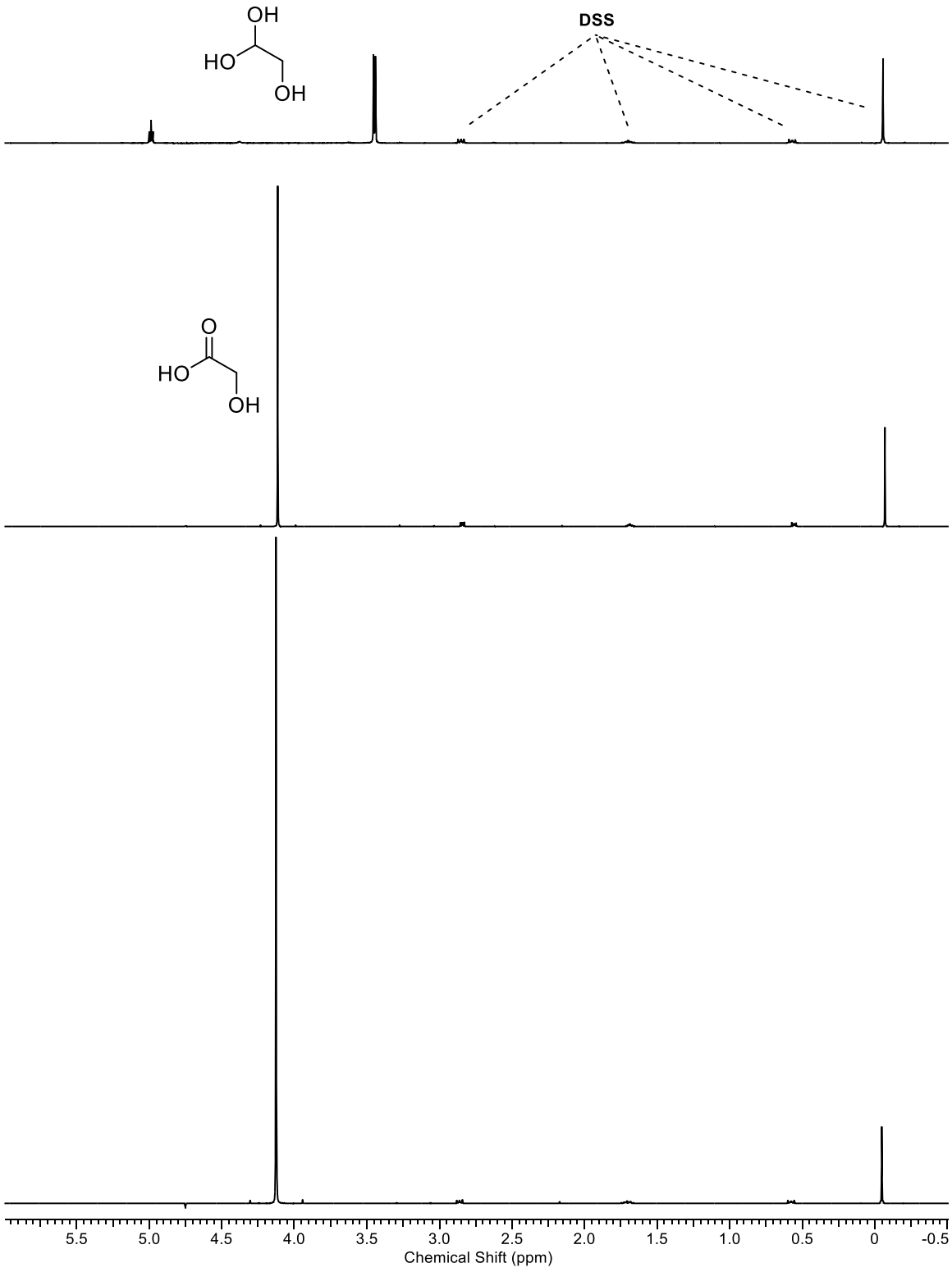


A91: ^{1}H NMR spectra (400 MHz, {60mM phosphate, D_2O}, 1.5–9.5 ppm) of **Top**, phosphoenolpyruvaldehyde (**105**, 70mM) and methionine (140mM) with DSS at pD 4 and **Bottom**, after incubation with sodium chlorite (98mM), 0 °C - ambient temperature, for 2h.

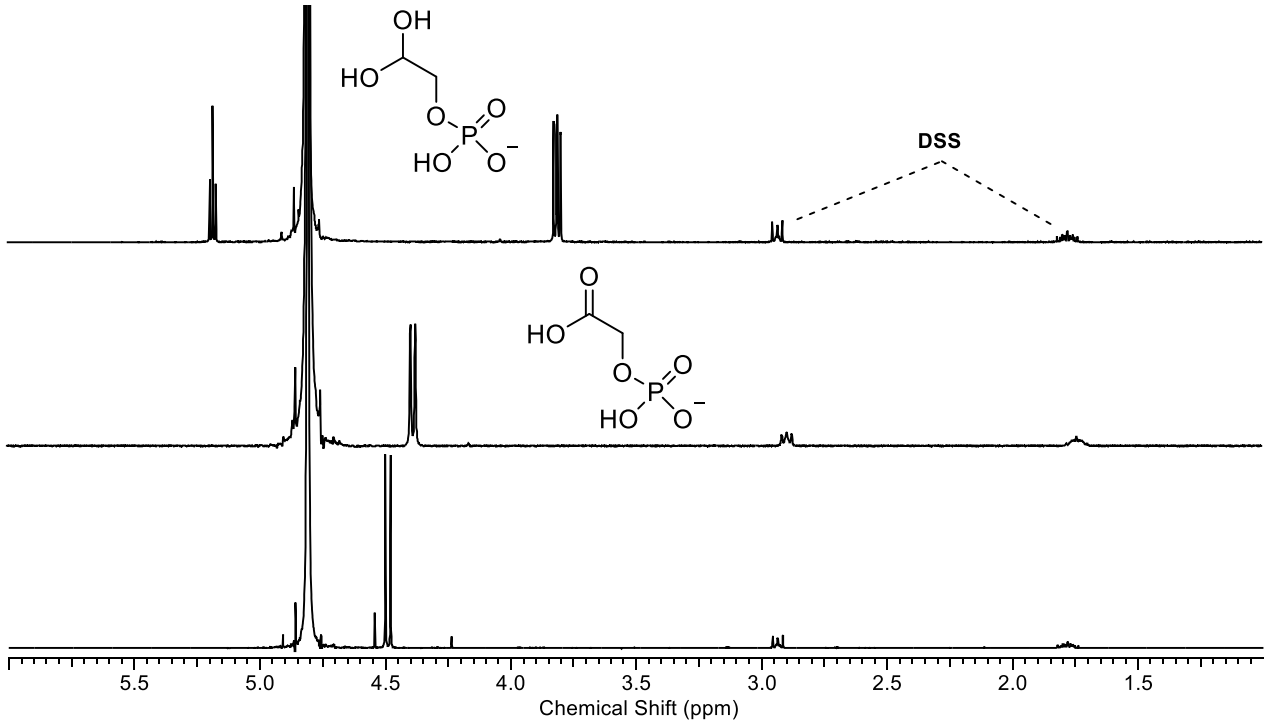




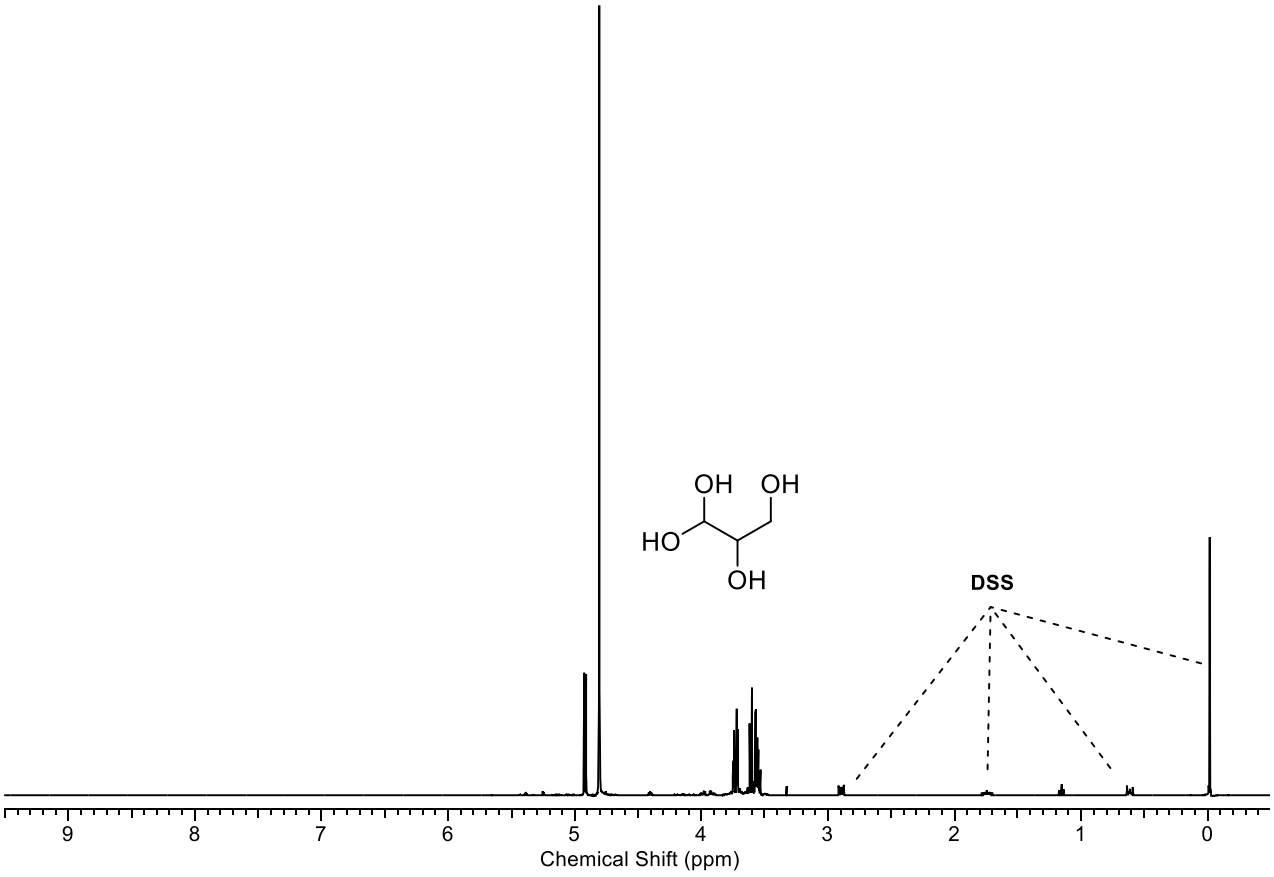
A92: ^{1}H NMR spectra (400 MHz, {60mM phosphate, D_2O}, -0.5 – 6.0 ppm) of **Top**, glycolaldehyde (**26**, 70mM) with DSS, **Middle**, after incubation with DMSO-d6 (140mM) and sodium chlorite (100mM), pD 4, 0 °C - ambient temperature, for 1.5 h and **Bottom**, after spiking with commercial glycolic acid, **45**.

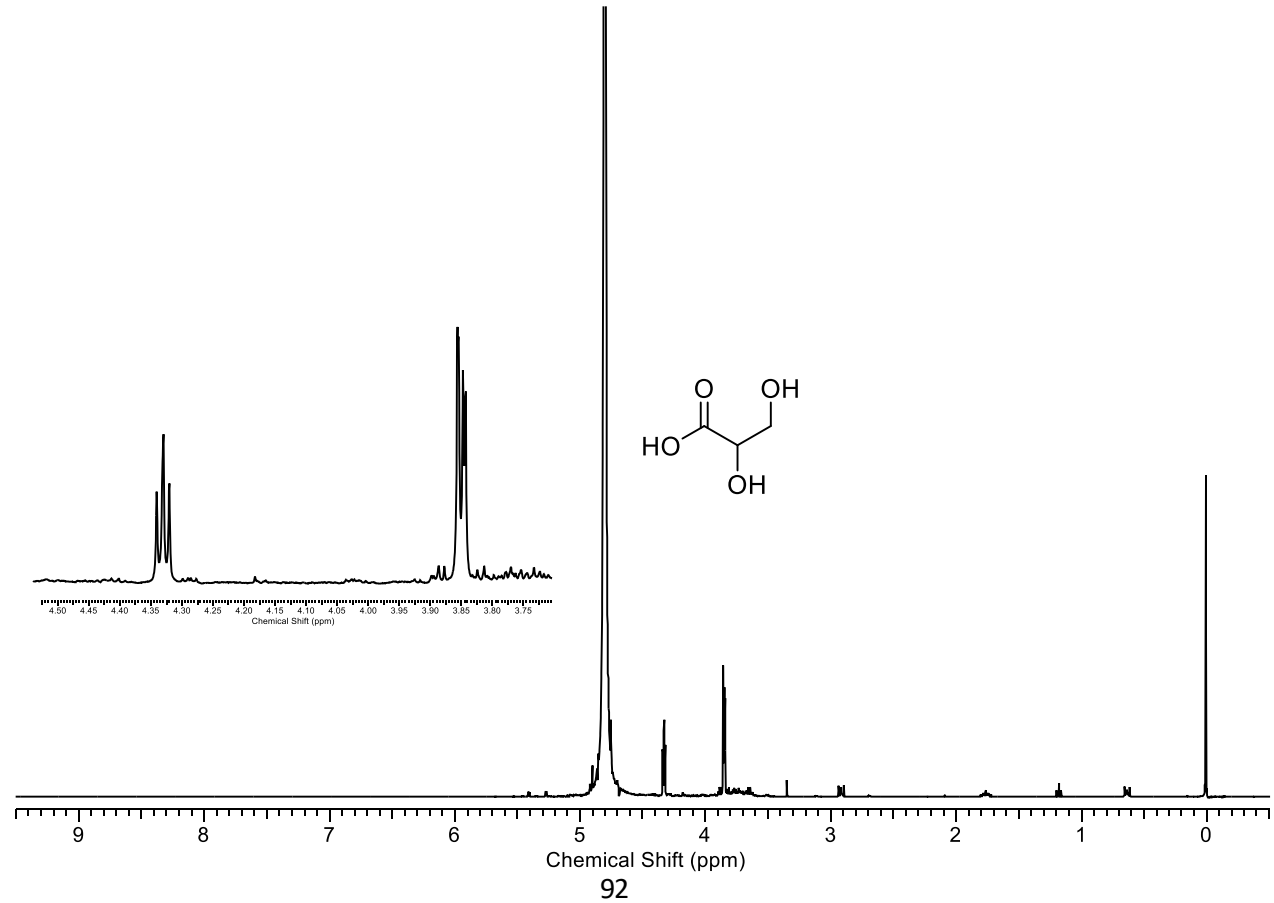


A93: ^{1}H NMR spectra (400 MHz, {60mM phosphate, D_2O}, 1.0 – 6.0 ppm) of **Top**, glycolaldehyde phosphate (**28**, 70mM) with DSS, **Middle**, after incubation with DMSO-d6 (140mM) and sodium chlorite (100mM), pD 4, 0 °C - ambient temperature, for 2 h and **Bottom**, after spiking with commercial glycolic acid phosphate, **46**.

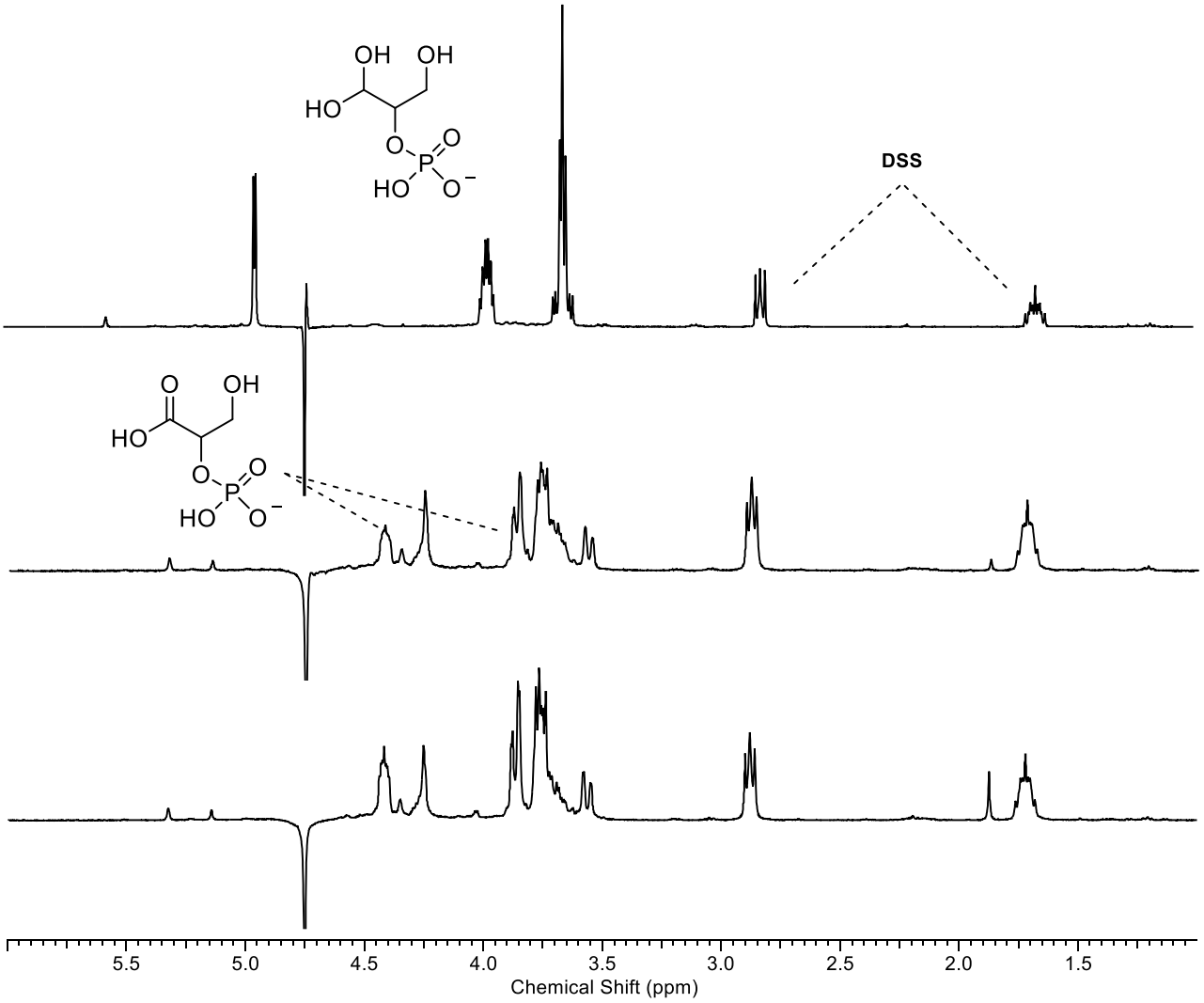


A94: ^{1}H NMR spectra (400 MHz, {60mM phosphate, D_2O}, -0.5 – 9.5 ppm) of **Top**, glyceraldehyde phosphate (**27**, 70mM) with DSS and **Bottom**, after incubation with DMSO-d6 (140mM) and sodium chlorite (100mM), pD 4, 0 °C - ambient temperature, for 2 h, with expansion overlaid.

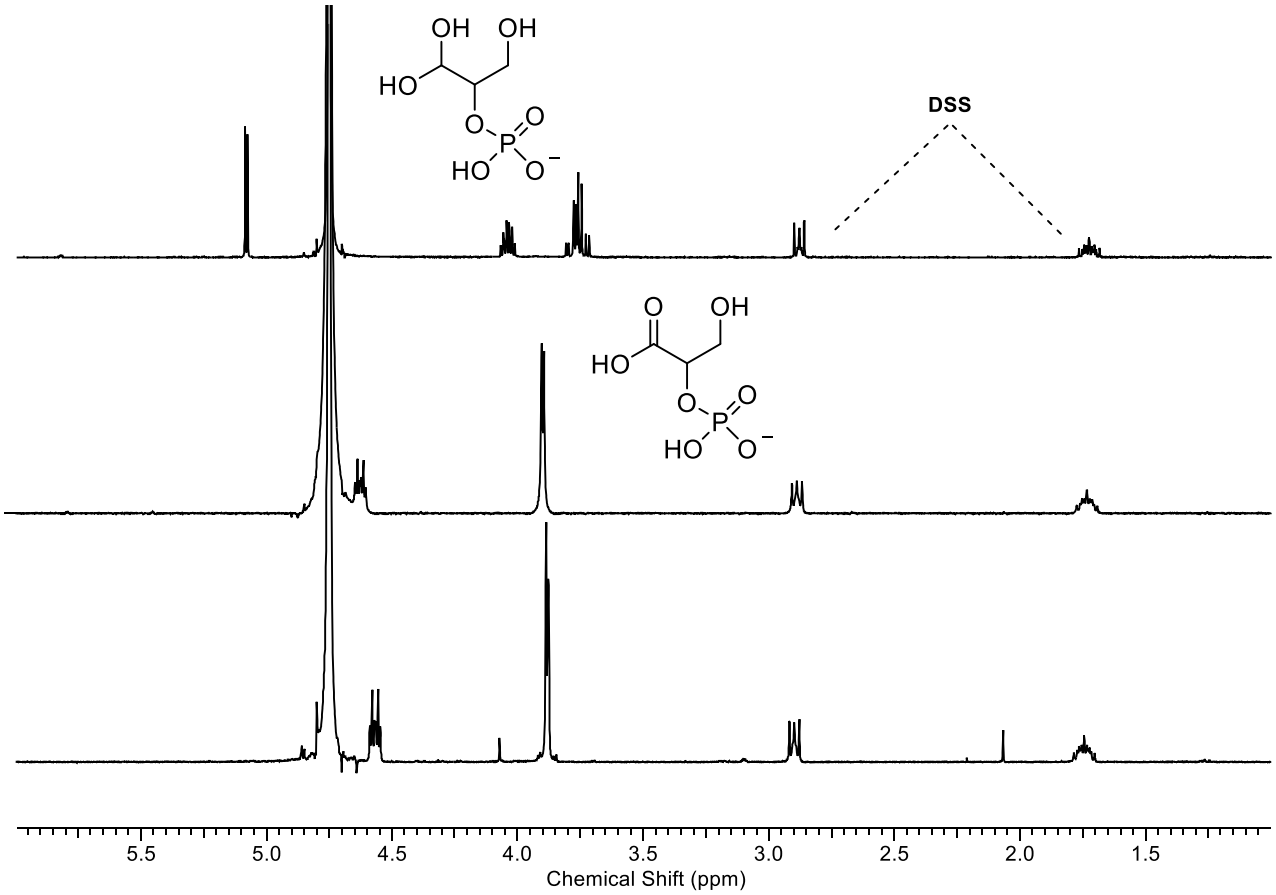




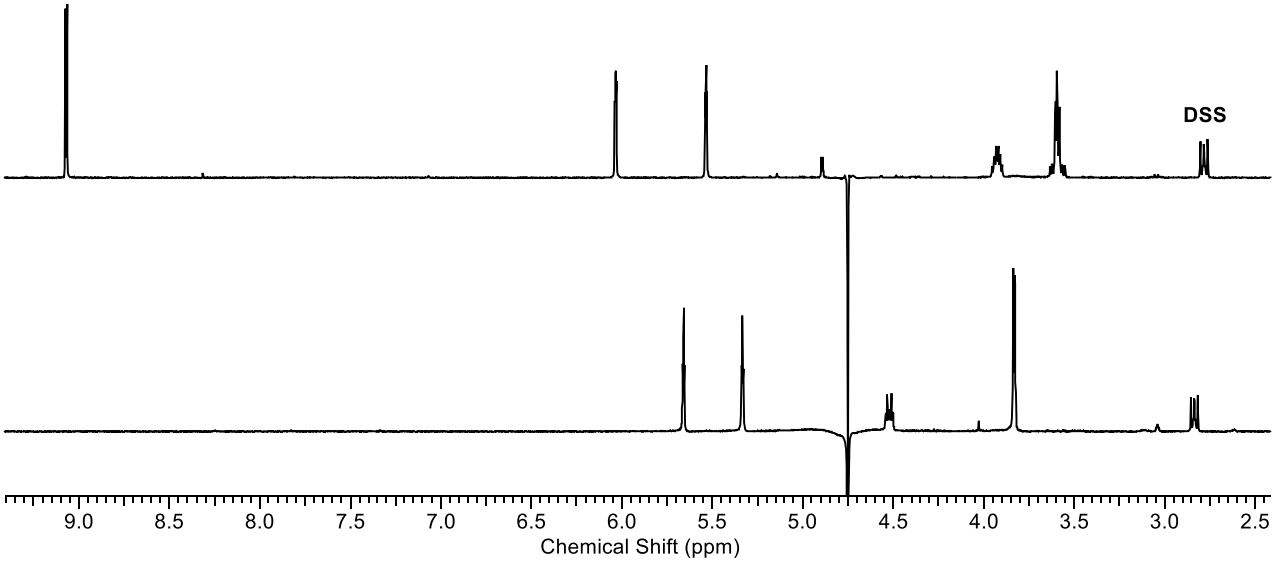
A95: ^{1}H NMR spectra (400 MHz, {H_2O/D_2O, 9:1}, 1.0 – 6.0 ppm) of **Top**, glyceraldehyde-2-phosphate (**29**, 100mM) with DSS, **Middle**, after incubation with sodium cyanide (500mM) and manganese dioxide (20 eq.) at pH 10.5, ambient temperature, for 2 h and **Bottom**, after spiking with commercial glyceric acid 2-phosphate, **43**.



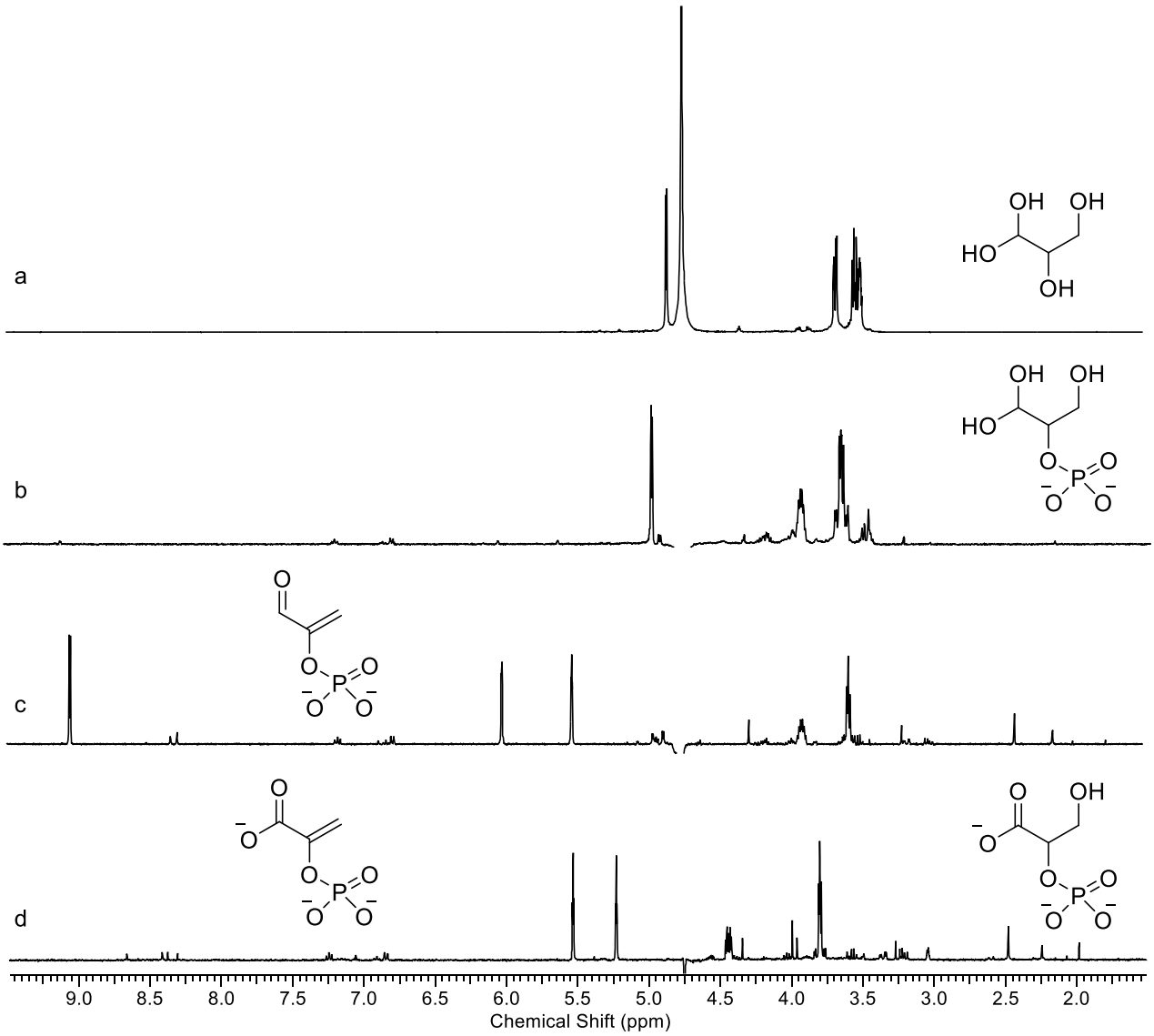
A96: ^{1}H NMR spectra (400 MHz, {60mM phosphate, D_2O}, 1.0 – 6.0 ppm) of **Top**, glyceraldehyde-2-phosphate (**29**, 70mM) with DSS, **Middle**, after incubation with DMSO-d6 (140mM) and sodium chlorite (100mM), pD 4, 0 °C - ambient temperature, for 0.5 h and **Bottom**, after spiking with commercial glycolic acid phosphate, **43**.

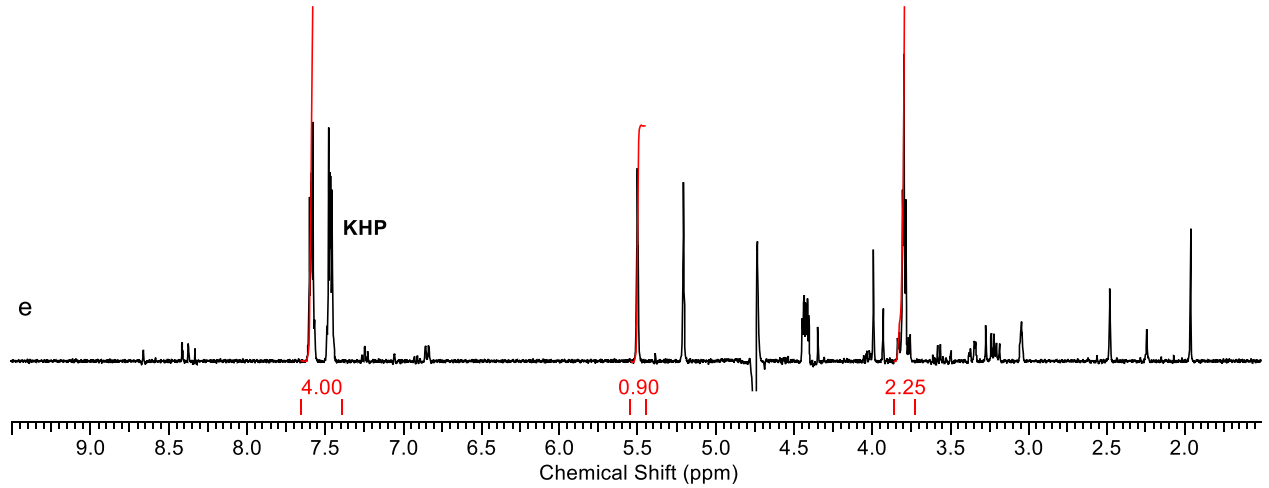


A97: ^{1}H NMR spectra (400 MHz, {500mM phosphate, H_2O/D_2O, 9:1}, 2.5 – 9.5 ppm) of **Top**, glyceraldehyde-2-phosphate (**29**, 63mM) with DSS after incubation at 60 °C, pH 7, 5 h and **Bottom**, after incubation with DMSO-d6 (126mM) and sodium chlorite (88mM), pH 4, 0 °C - ambient temperature, for 2 h.



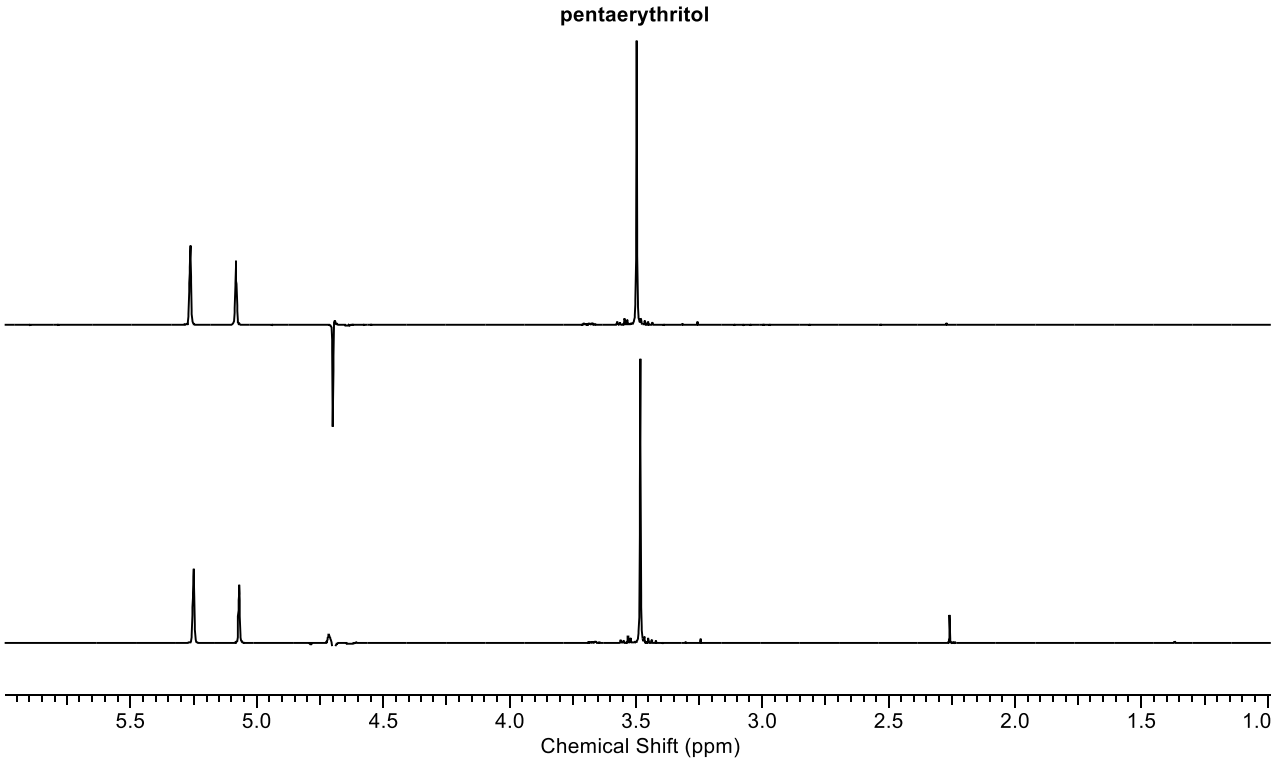
A98: ^{1}H NMR spectra (400 MHz, {D_2O [**a**] or 750mM phosphate, H_2O/D_2O, 9:1 [**b**, **c**, **d**, **e**]}, 1.5 – 9.5 ppm) of **a**, glyceraldehyde, **27**; **b**, the reaction of **27** (25mM) with diamidophosphate (**48**, 4 eq.) at pH 4, ambient temperature for 4 h; **c**, after adjustment to pH 7 and incubation at 60 °C for 4 h; **d**, after readjustment to pH 4 and incubation with DMSO-d6 (2 eq.) and sodium chlorite (1.4 eq.) at 0 °C - ambient temperature over 2 h and **e**, showing the use of potassium hydrogen phthalate (KHP), added after completion of the reaction, to quantify the product conversion.


a
b
c
d
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Chemical Shift (ppm)

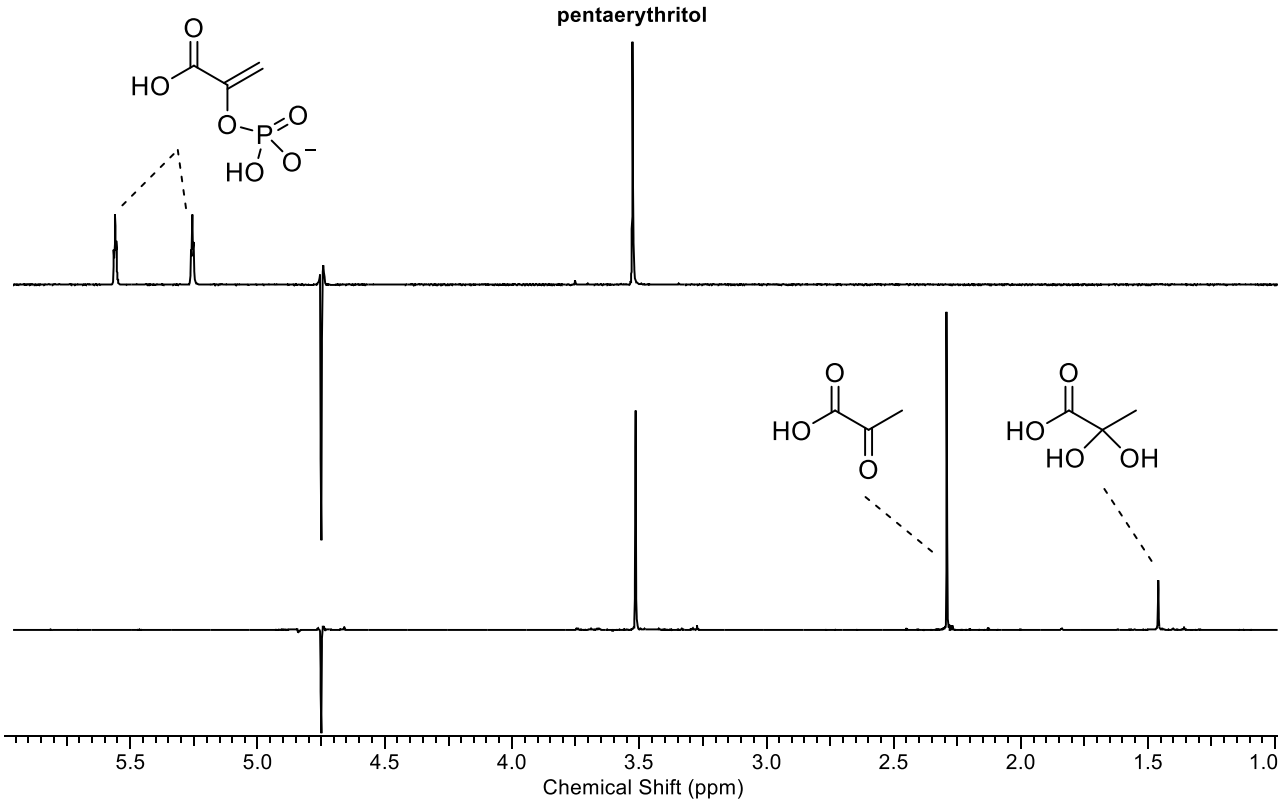

KHP
e
4.00
0.90
2.25
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Chemical Shift (ppm)

Pyruvate (**64**)

A99: ^{1}H NMR spectra (400 MHz, {H_2O/D_2O, 9:1}, 1.0 – 6.0 ppm) of **Top**, phosphoenol pyruvate (**94**, 63mM) with pentaerythritol and **Bottom**, after incubation at 60 °C, pH 9.5, for 50 h.

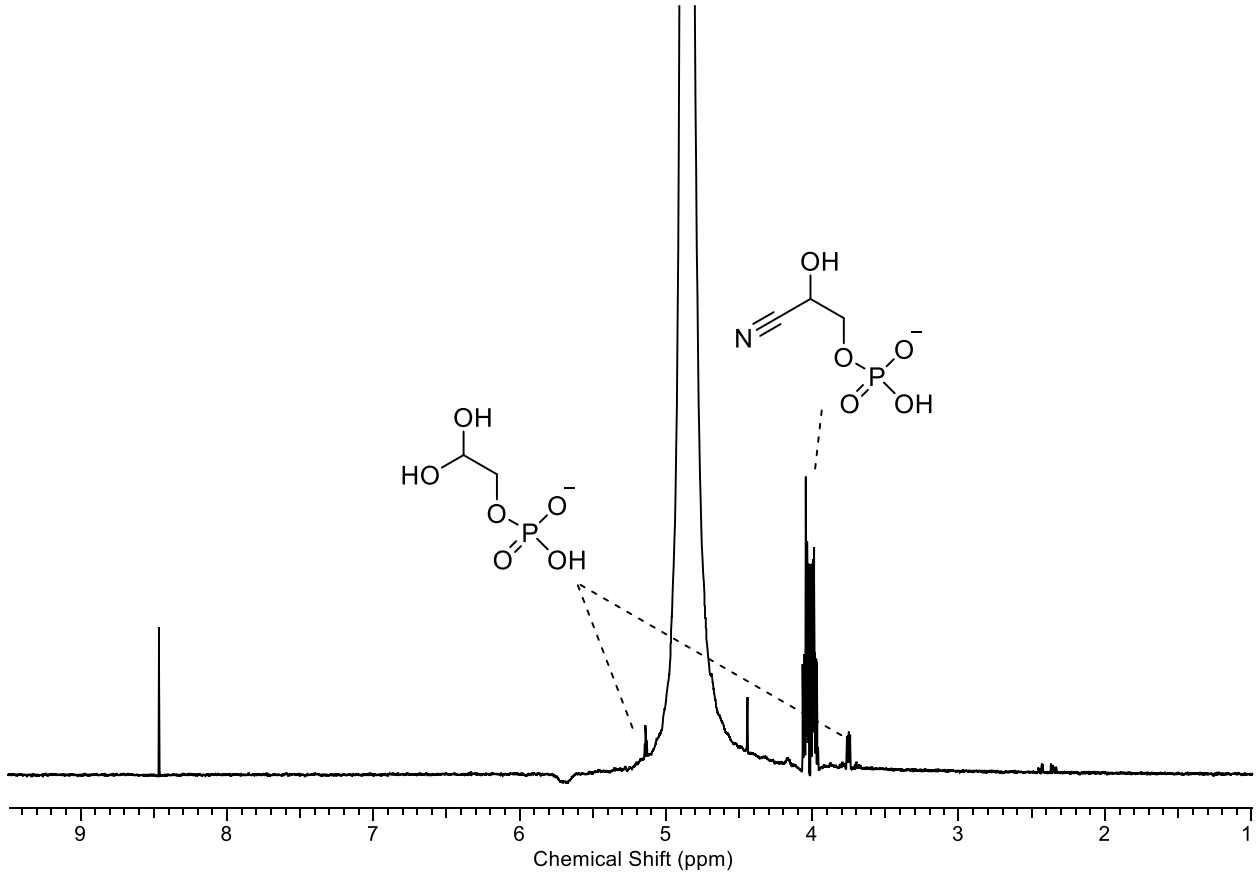


A100: ^{1}H NMR spectra (400 MHz, {H_2O/D_2O, 9:1}, 1.0 – 6.0 ppm) of **Top**, phosphoenol pyruvate (**94**, 63mM) with pentaerythritol and **Bottom**, after incubation at 60 °C, pH 4, for 50 h.

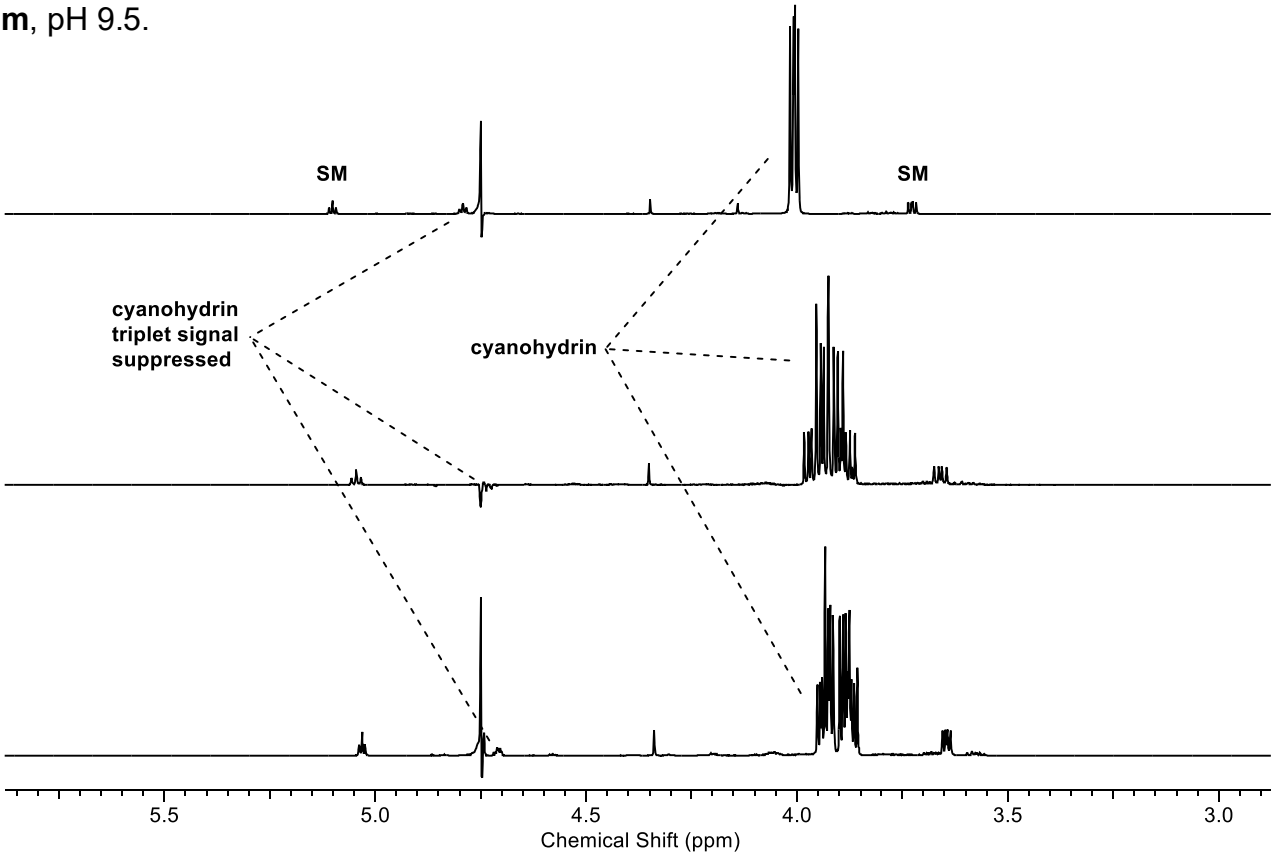


Glycolaldehyde phosphate cyanohydrin (**126**)

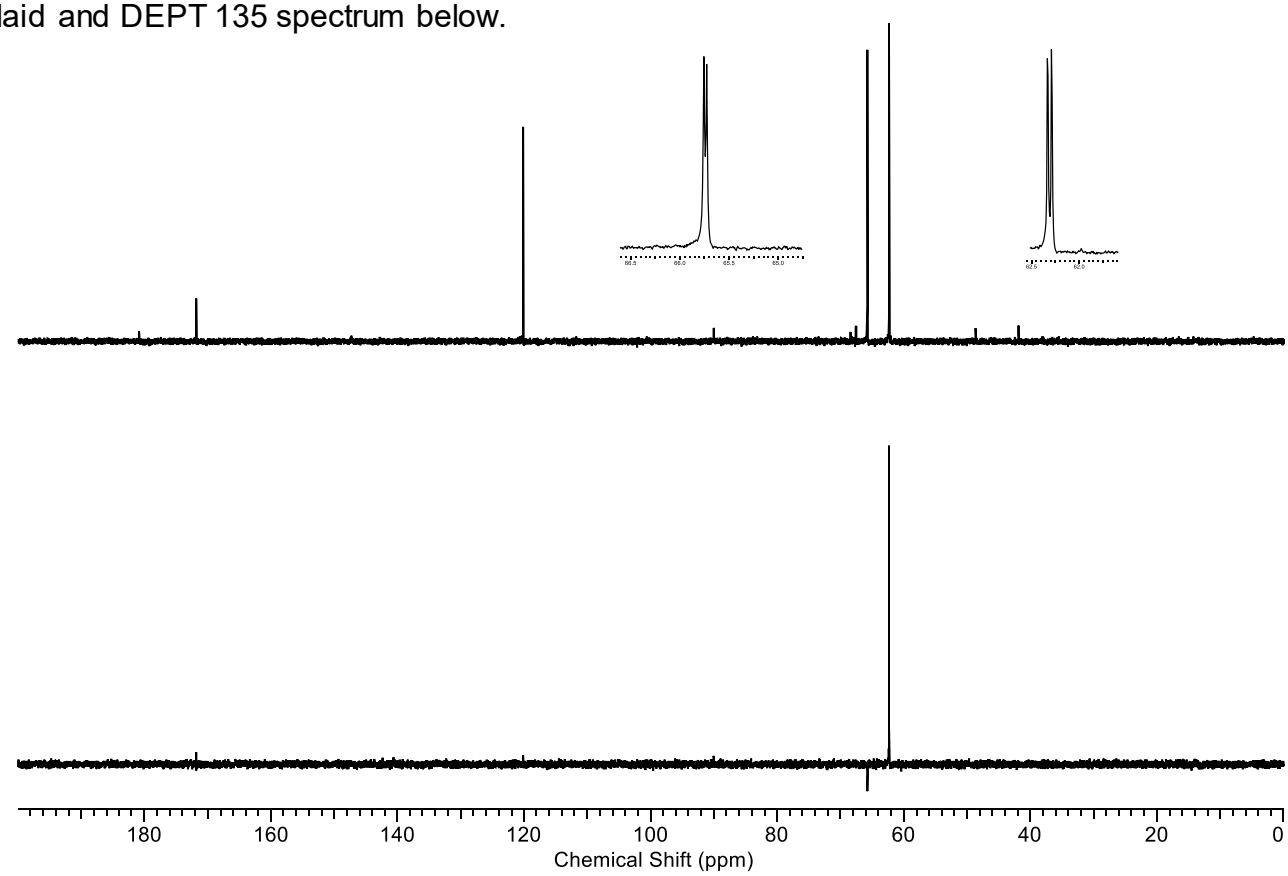
A101: ^{1}H NMR spectrum (600 MHz, {H_2O/D_2O, 9:1}, 1.0 – 9.5 ppm) of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (240mM) at ambient temperature, pH 7 (no solvent suppression).



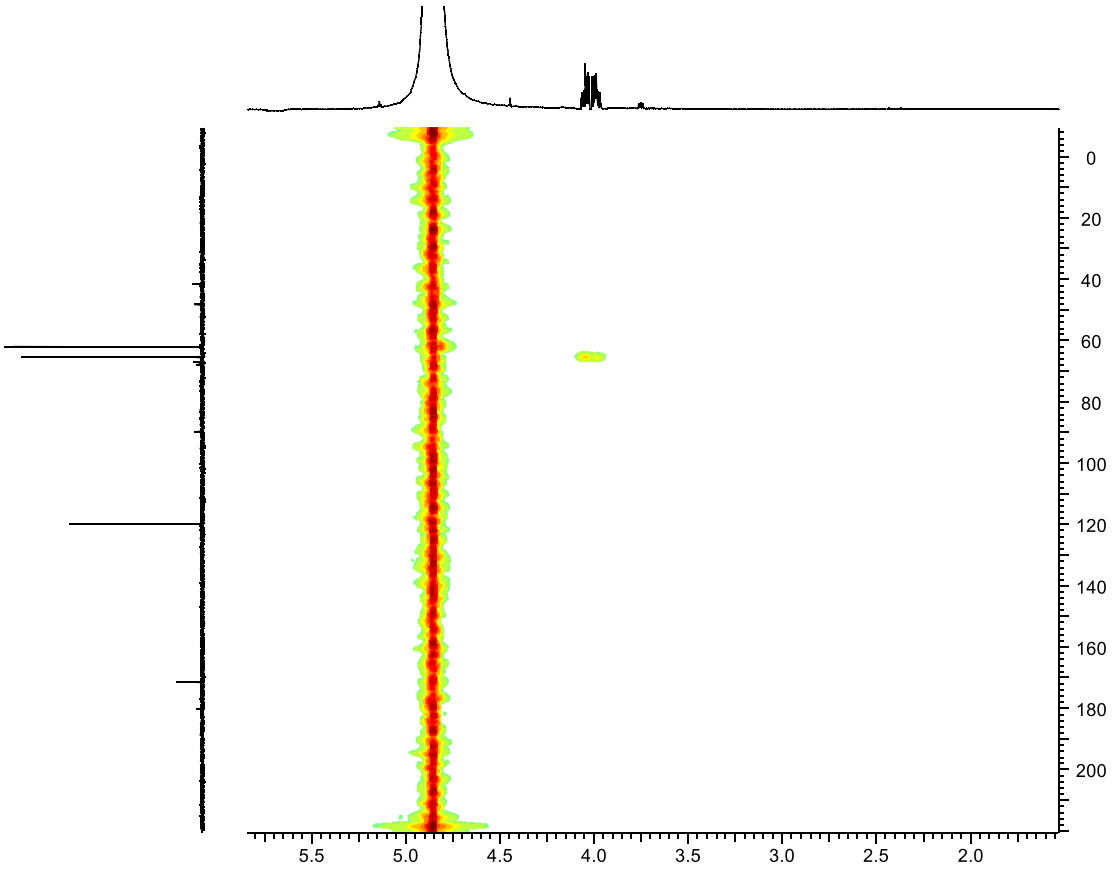
A102: ^{1}H NMR spectra (600 MHz, {H_2O/D_2O, 9:1}, 3.9 – 5.9 ppm) of glycolaldehyde phosphate **28** (200mM) and sodium cyanide (240mM) at ambient temperature, **Top**, pH 2, **Middle**, pH 7 and **Bottom**, pH 9.5.



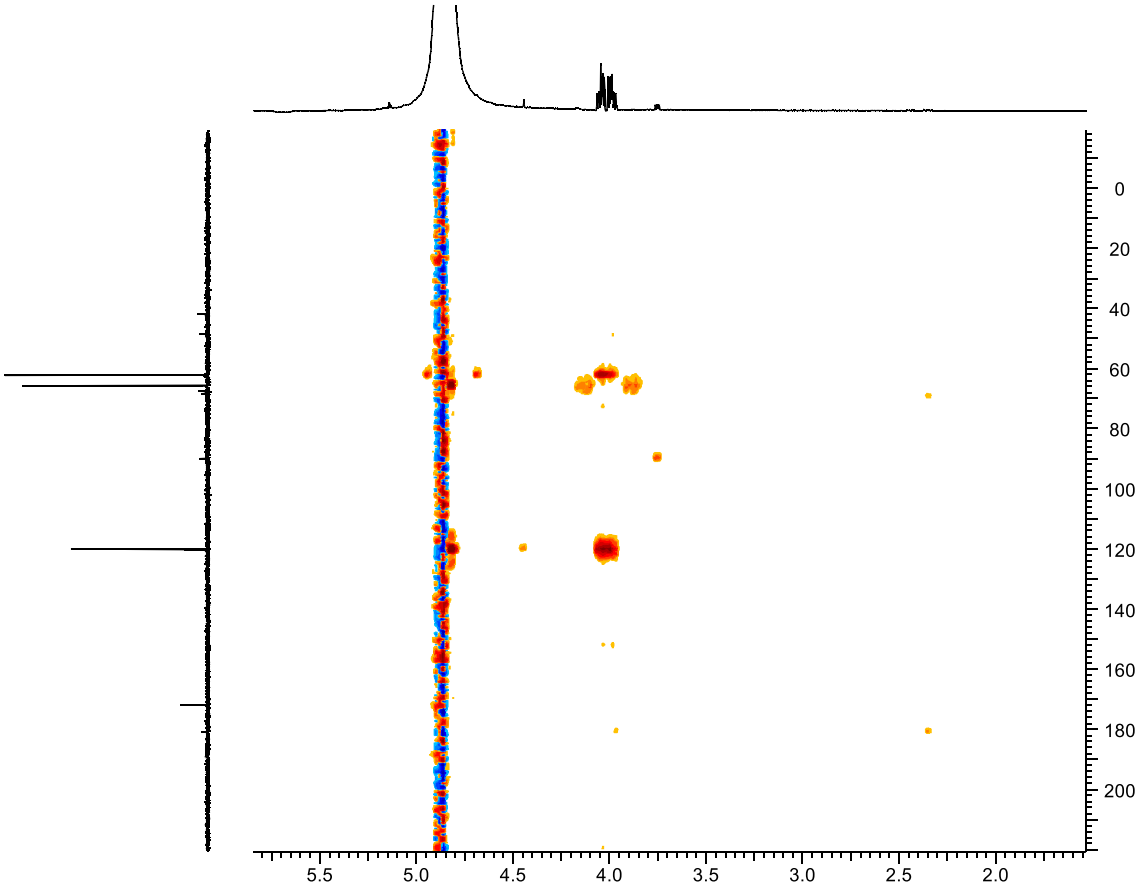
A103: ^{13}C NMR spectrum (151 MHz, {H_2O/D_2O, 9:1}, 0 – 200 ppm) of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (240mM) at ambient temperature, pH 7, with expanded peaks overlaid and DEPT 135 spectrum below.



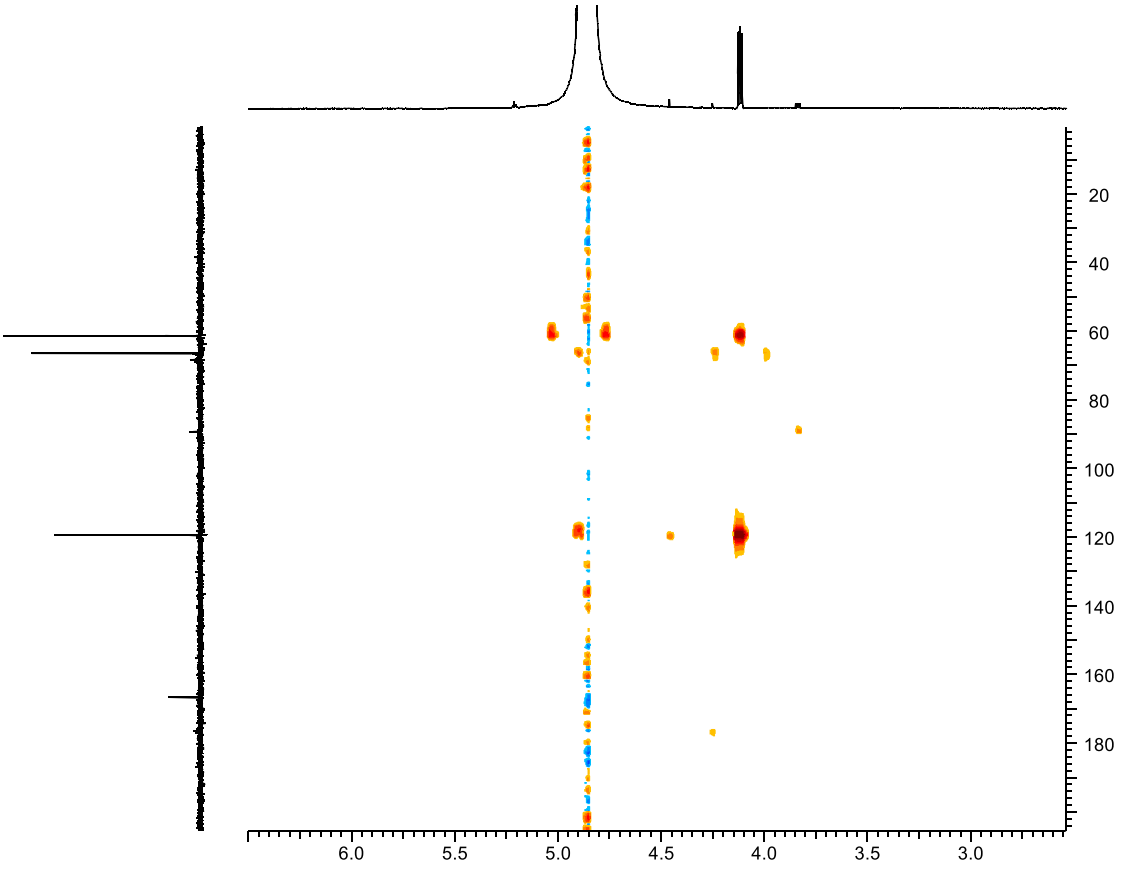
A104: ^{1}H-^{13}C HSQC NMR spectrum (600 MHz, {H_2O/D_2O, 9:1}) of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (240mM) at ambient temperature, pH 7.



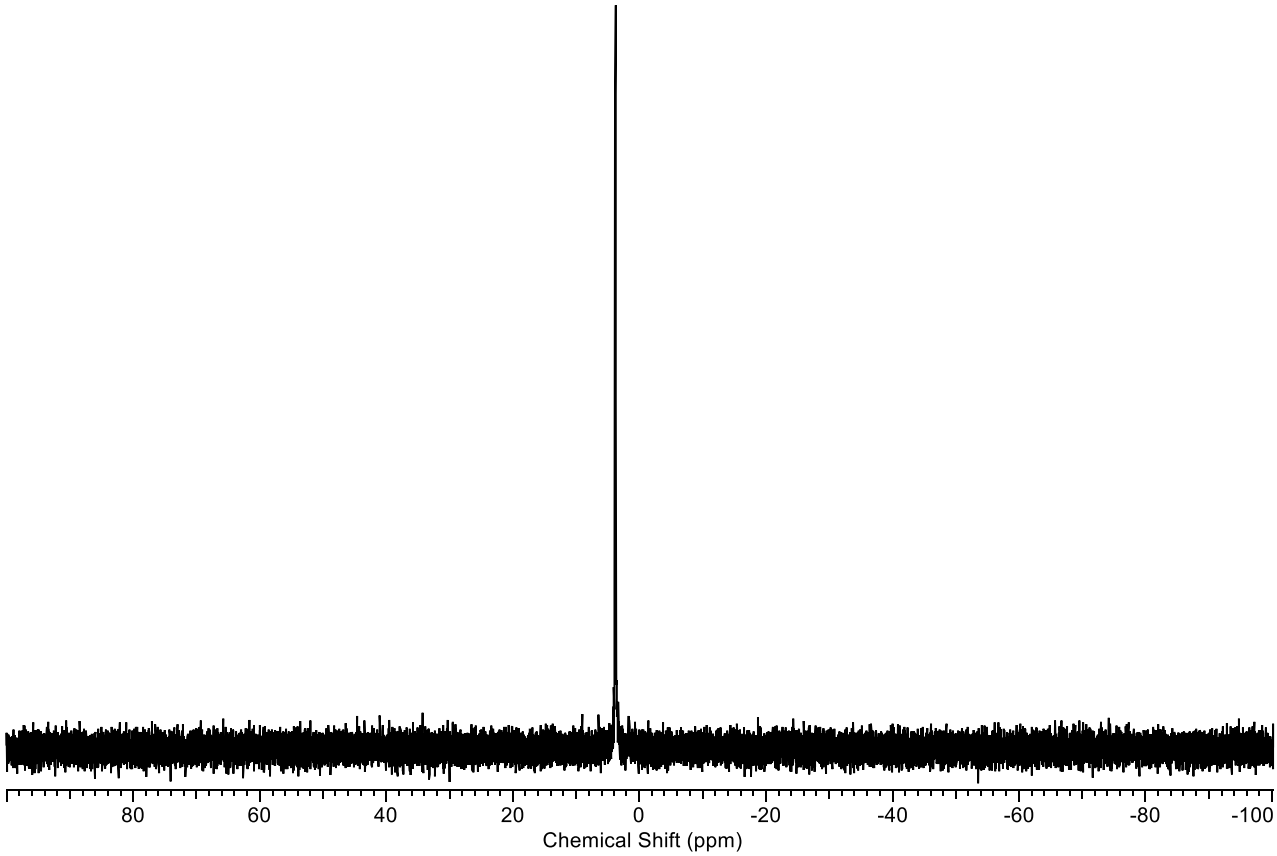
A105: ^{1}H-^{13}C HMBC NMR spectrum (600 MHz, {H_2O/D_2O, 9:1}) of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (240mM) at ambient temperature, pH 7.



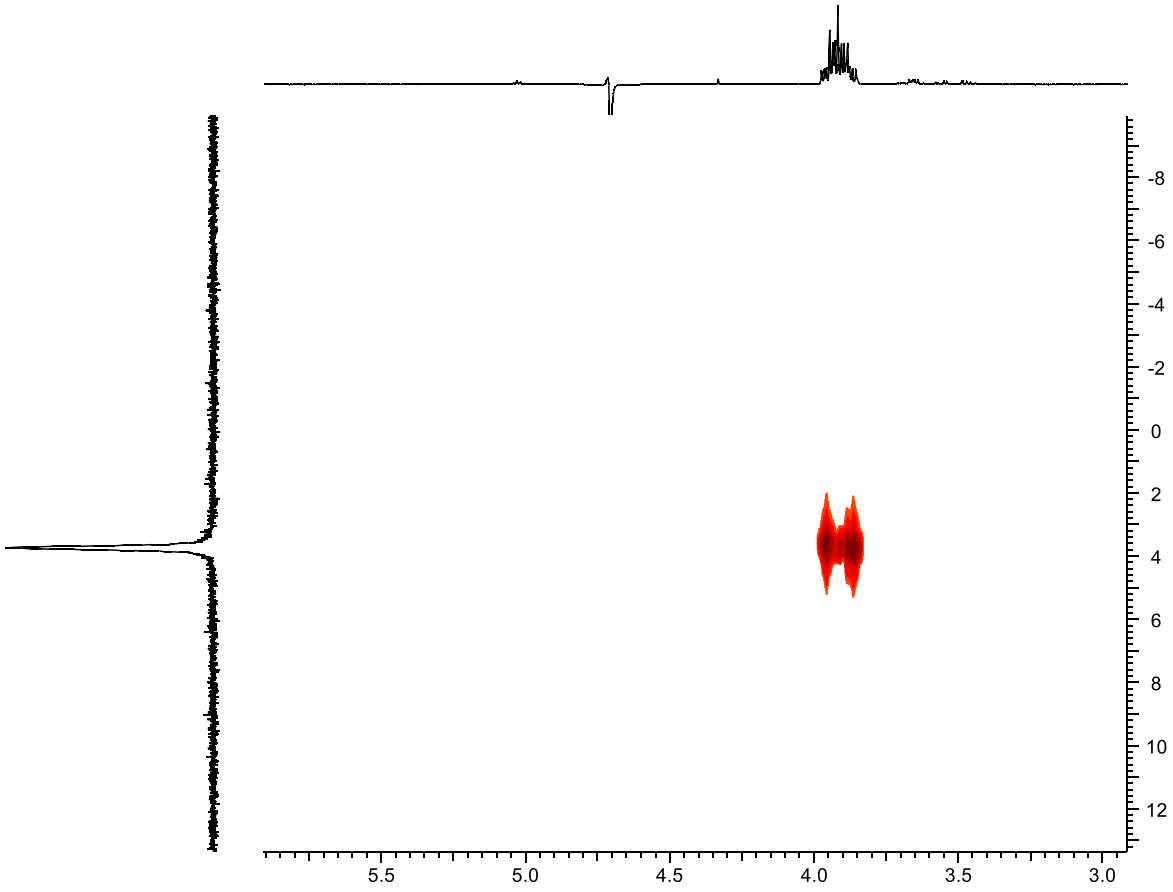
A106: ^{1}H-^{13}C HMBC NMR spectrum (600 MHz, {H_2O/D_2O, 9:1}) of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (240mM) at ambient temperature, pH 2.



A107: ^{31}P NMR spectrum (161 MHz, {H_2O/D_2O, 9:1} -100 – 100 ppm) of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (240mM) at ambient temperature, pH 7.

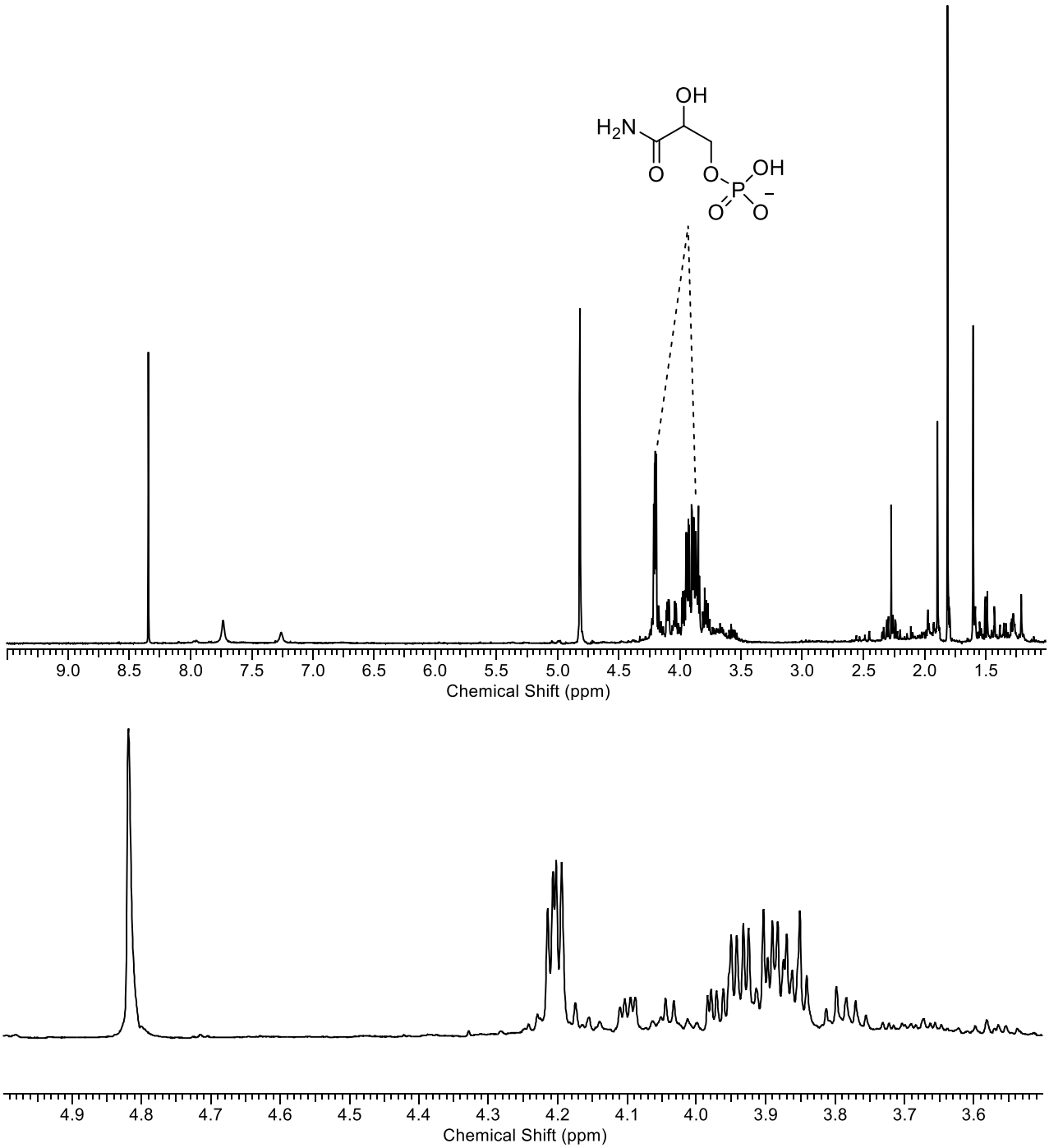


A108: ^{1}H-^{31}P HMBC NMR spectrum (400 MHz, {H_2O/D_2O, 9:1}) of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (240mM) at ambient temperature, pH 7.

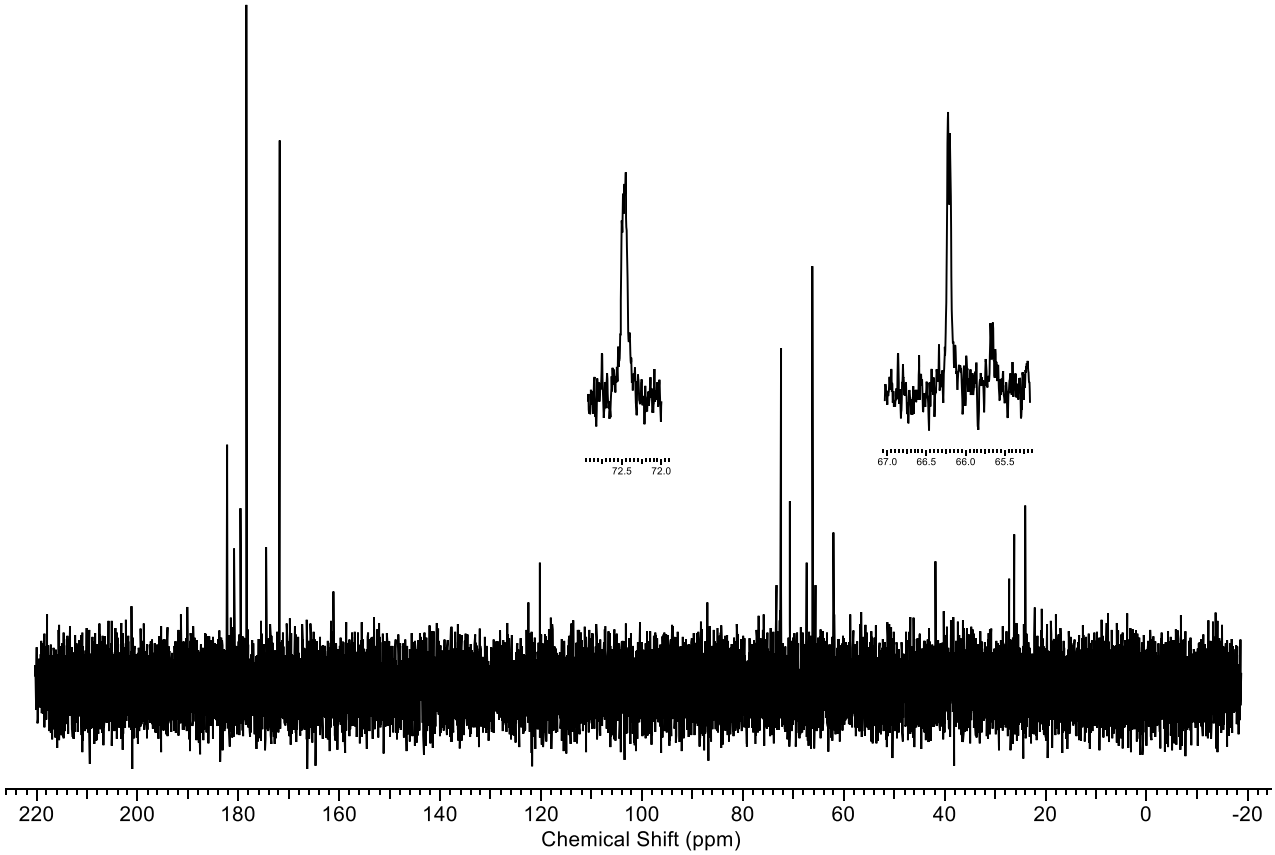


Glyceramide-3-phosphate (**127**)

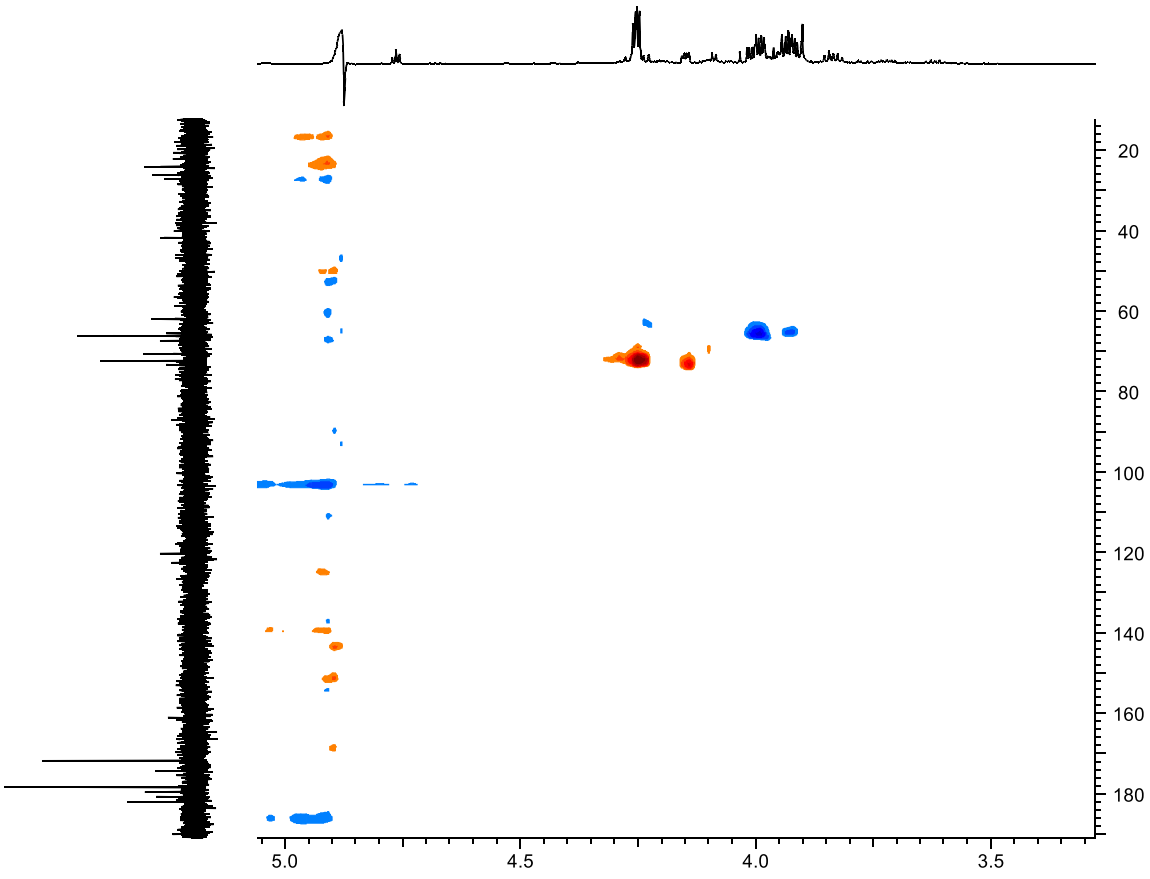
A109: ^{1}H NMR spectrum (600 MHz, {750mM phosphate, H_2O/D_2O, 9:1}, 1.0 – 9.5 ppm) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (233mM) at 75 °C, pH 7, 6 days with expansion below.



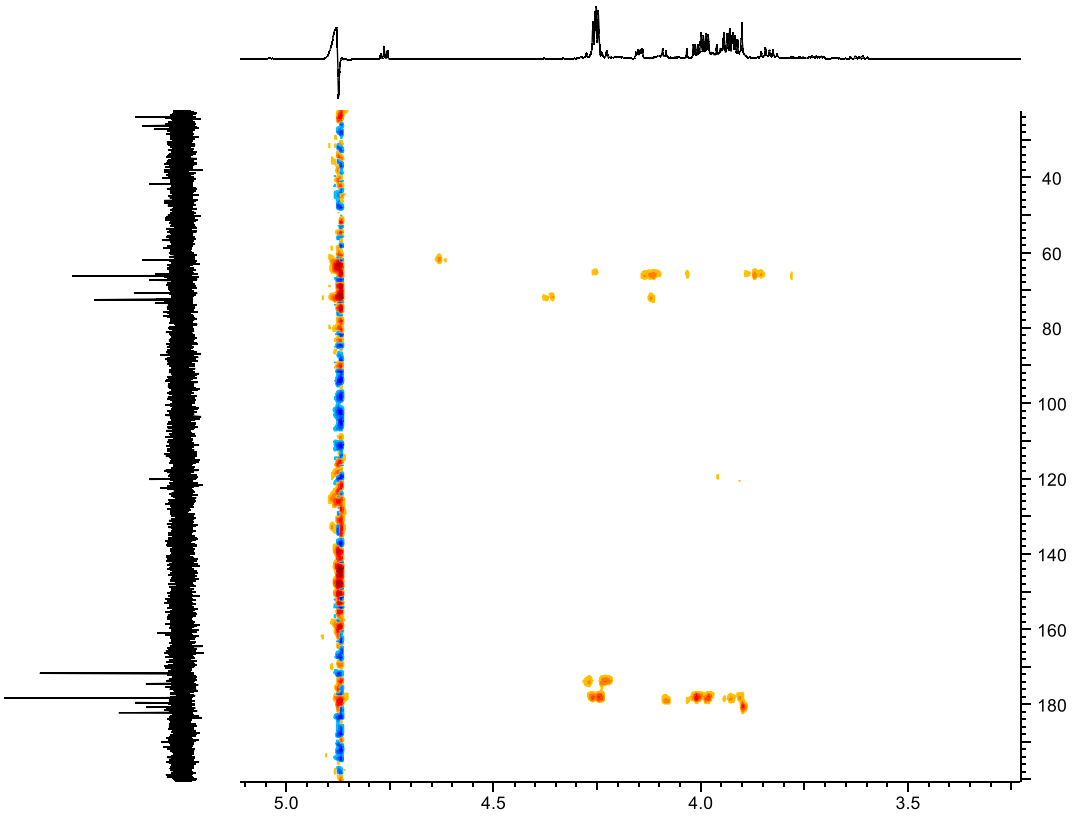
A110: ^{13}C NMR spectrum (151 MHz, {750mM phosphate, H_2O/D_2O, 9:1}, -20 – 220 ppm) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (240mM) at 75 °C, pH 7, 6 days, with expanded peaks overlaid.



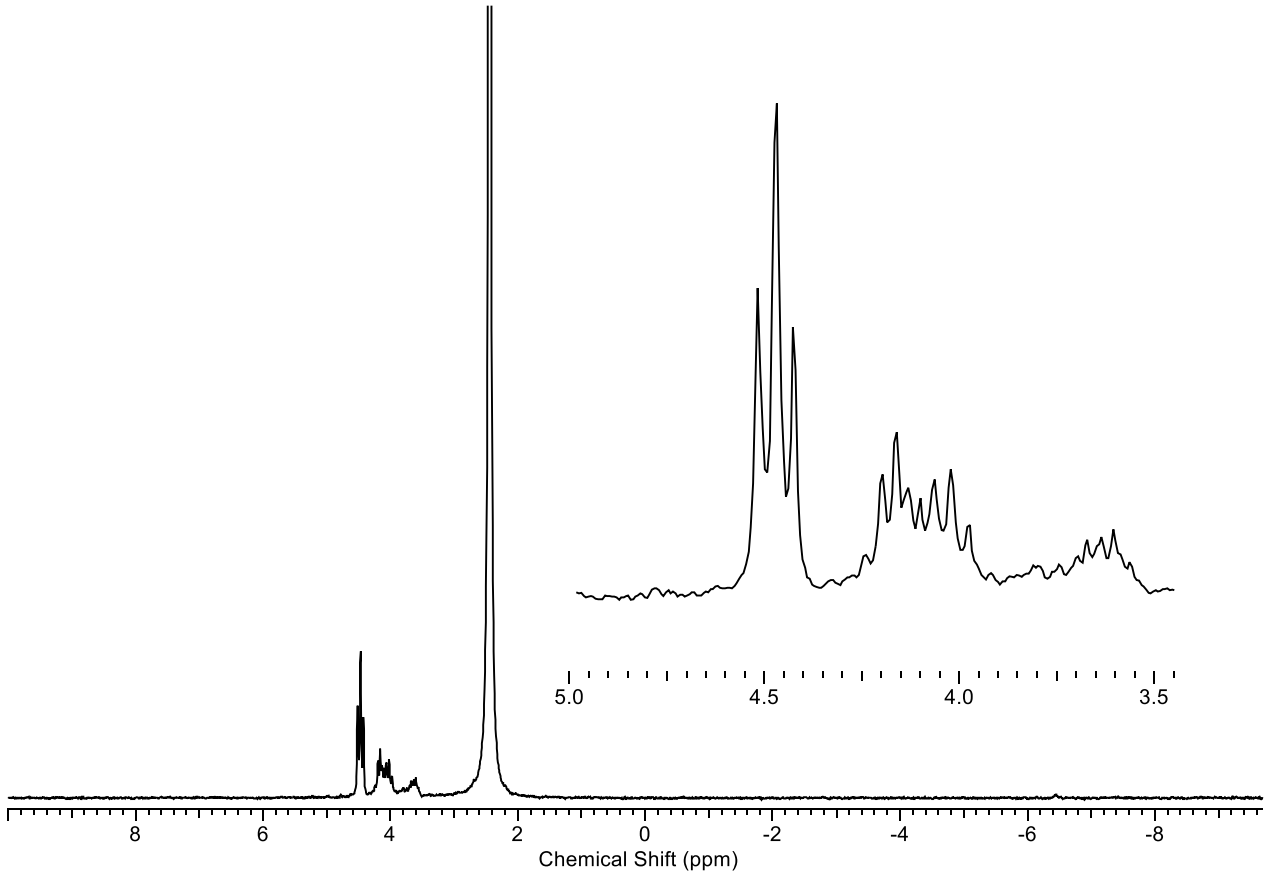
A111: ^{1}H-^{13}C HSQC NMR spectrum (600 MHz, {750mM phosphate, H_2O/D_2O, 9:1}) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (240mM) at 75 °C, pH 7, 6 days.



A112: ^{1}H-^{13}C HMBC NMR spectrum (600 MHz, {750mM phosphate, H_2O/D_2O, 9:1}) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (240mM) at 75 °C, pH 7, 6 days.

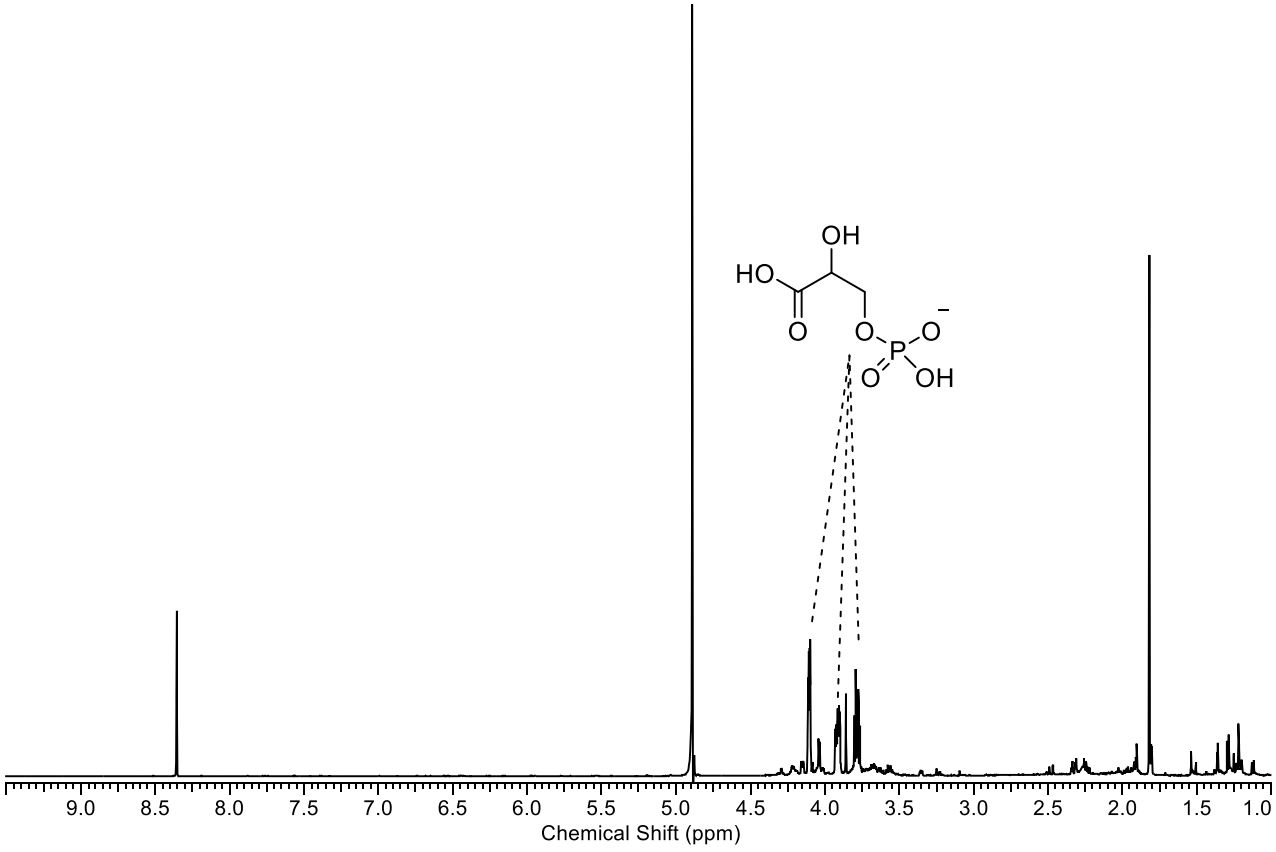


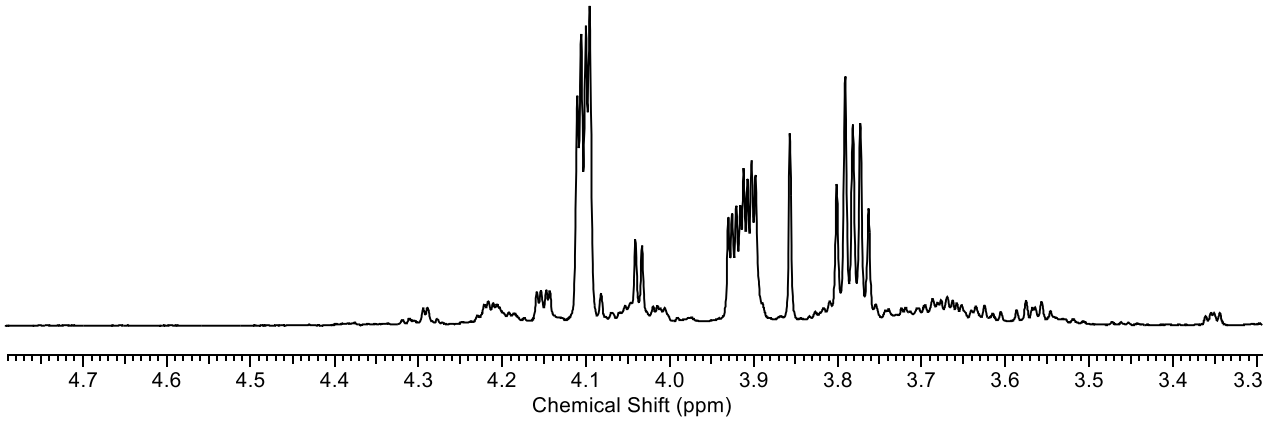
A113: ^{31}P NMR spectrum (161 MHz, {750mM phosphate, H_2O/D_2O, 9:1} -10 – 10 ppm) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (240mM) at 75 °C, pH 7, 6 days, with expansion overlaid.



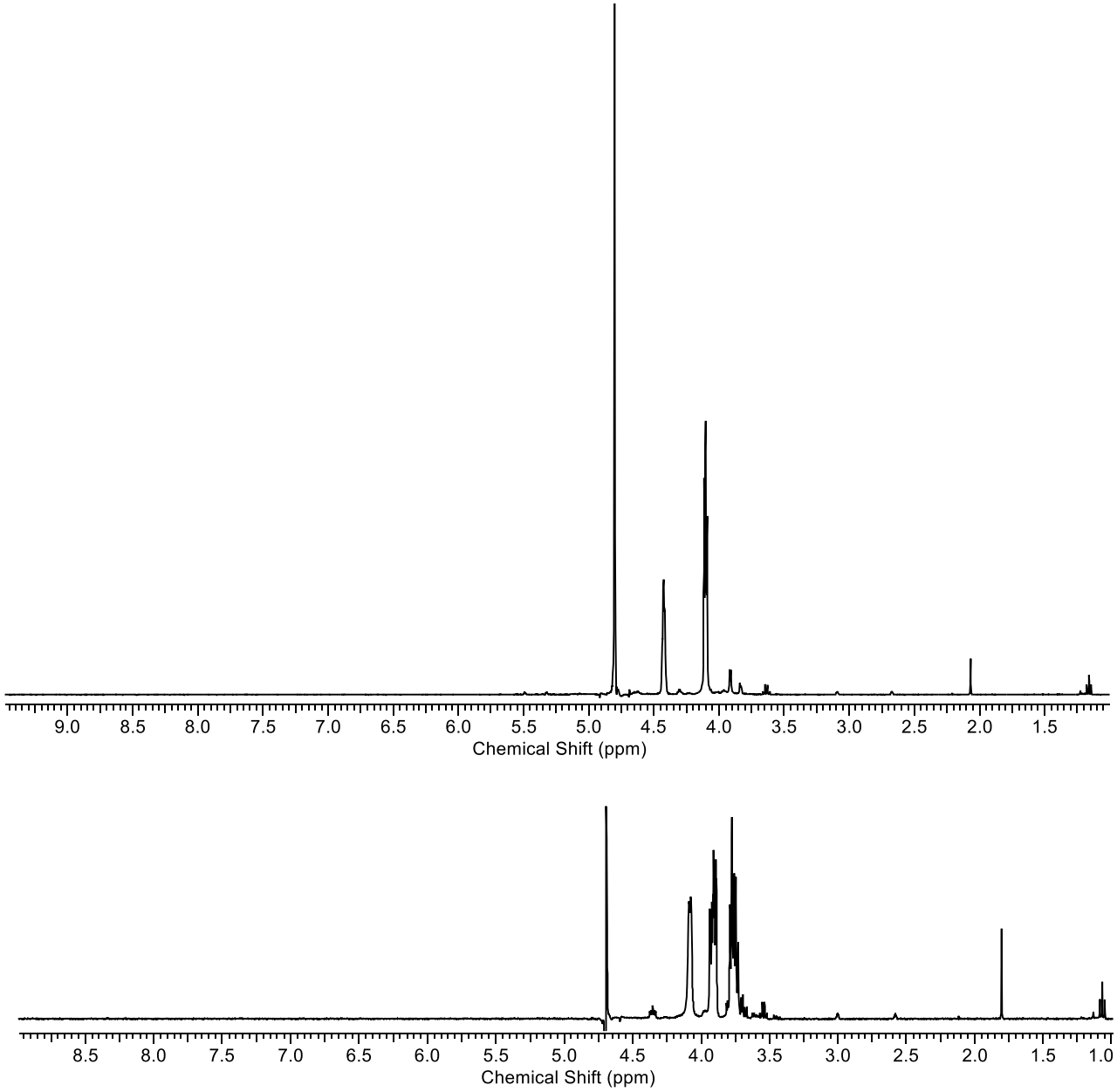
Glyceric acid-3-phosphate (**44**)

A114: ^{1}H NMR spectrum (600 MHz, {750mM phosphate, H_2O/D_2O, 9:1}, 1.0 – 9.5 ppm) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (233mM) at 75 °C, pH 7, 6 days followed by incubation at 75 °C, pH 12, 5 h, with expansion below.

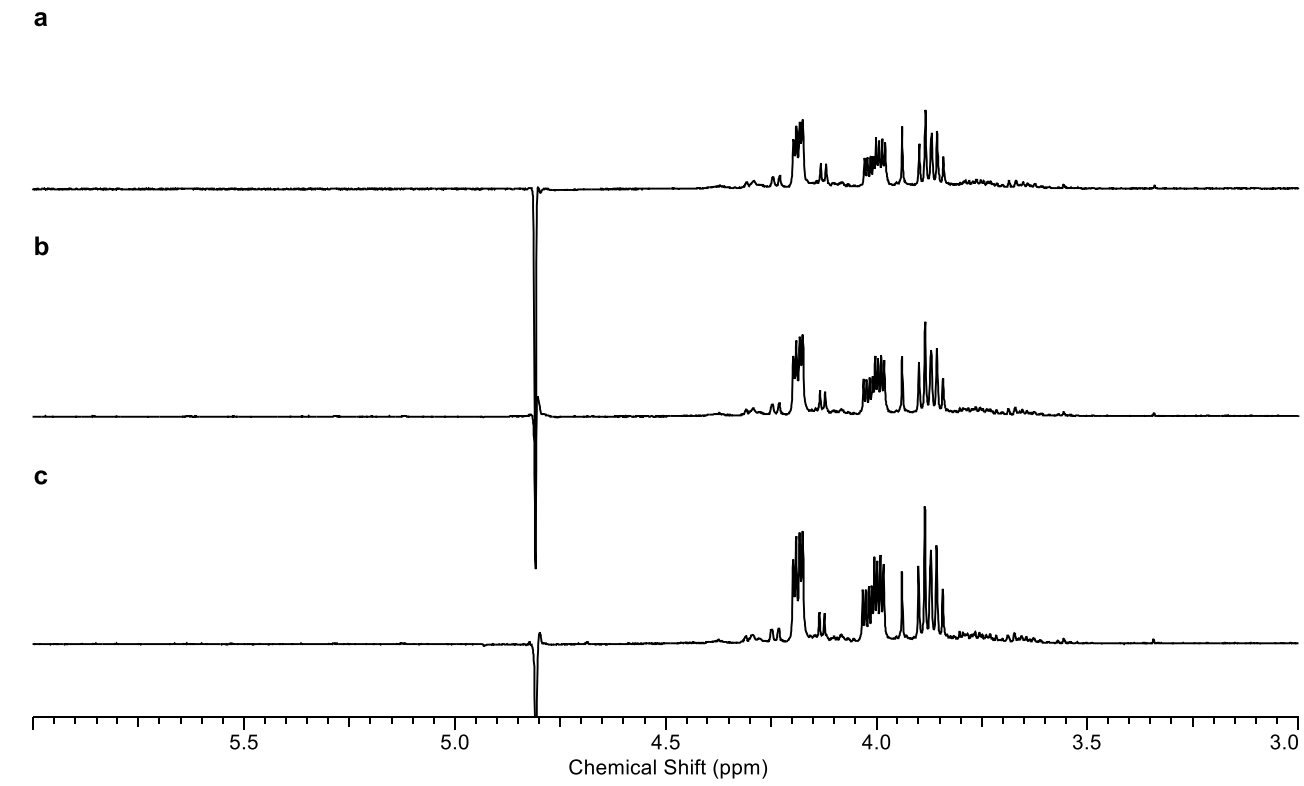


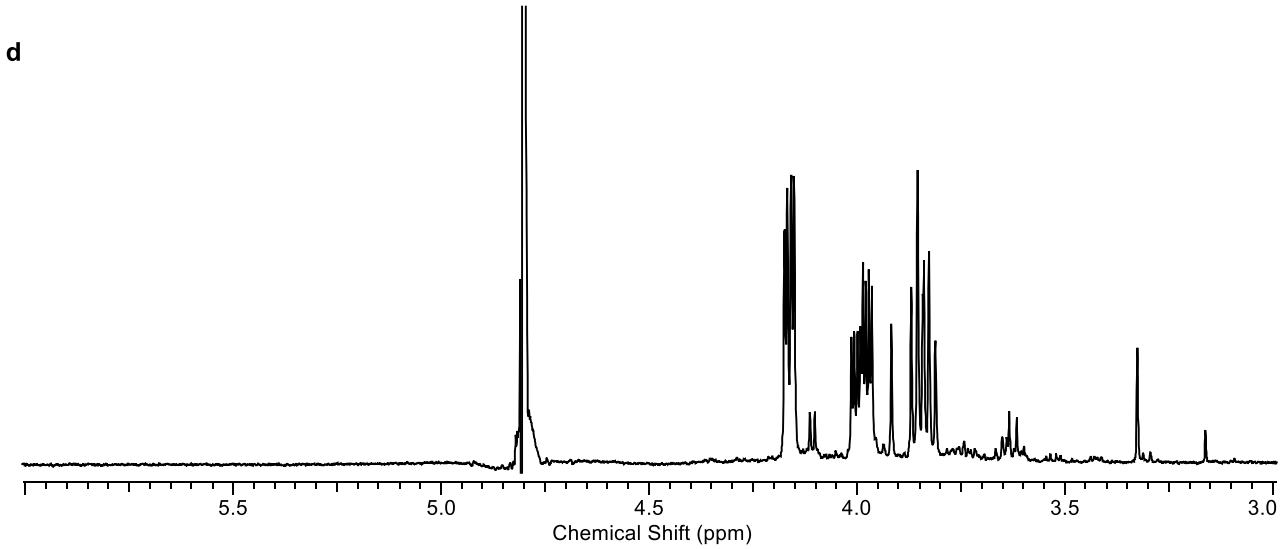


A115: ^{1}H NMR spectra (400 MHz, {H_2O/D_2O, 9:1}, 1.0 – 9.5 ppm) of **Top**, the reaction of glycolaldehyde-3-phosphate (**93**, 70mM) and sodium chlorite (98mM) at 0 °C, pH 4, 0.5 h and **Bottom**, after adjustment to pH 10.

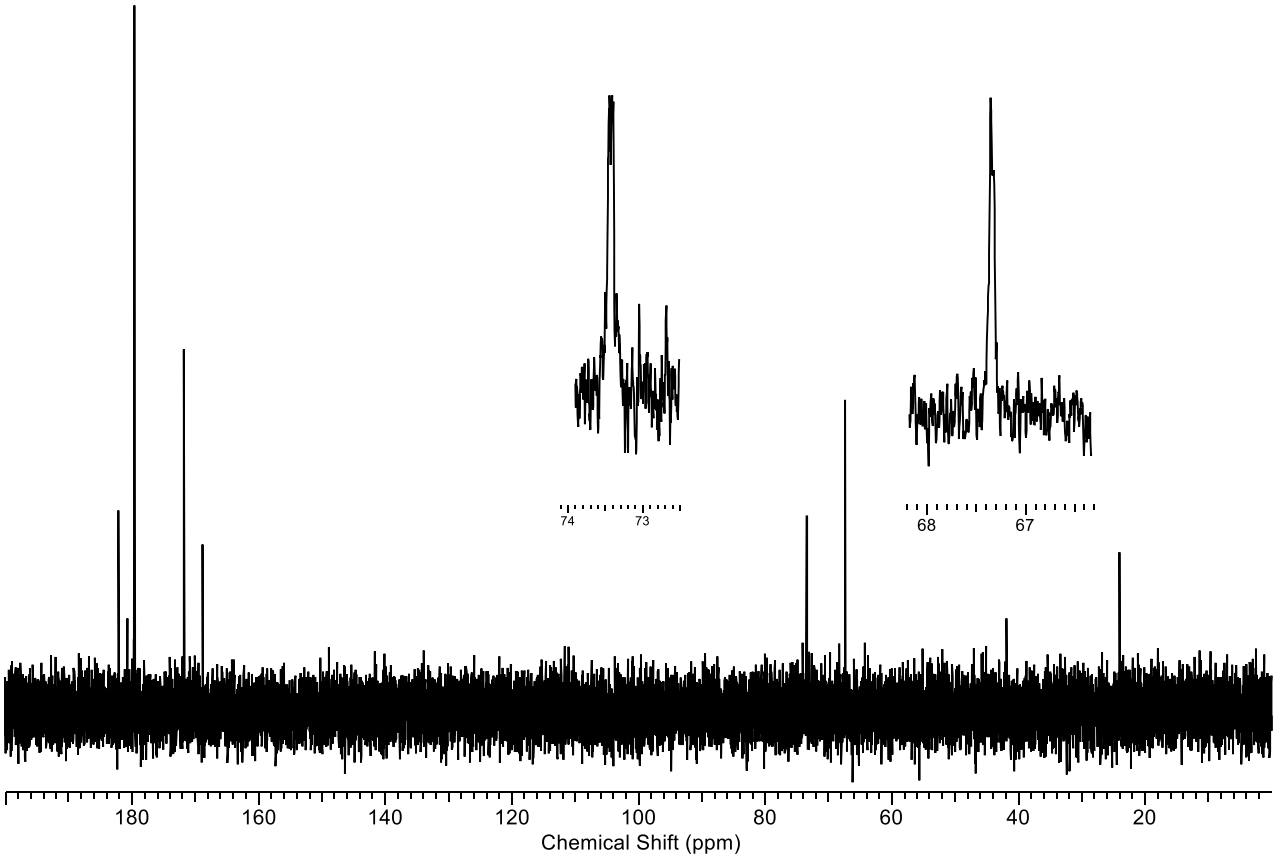


A116: ^{1}H NMR spectra (400 MHz, {H_2O/D_2O, 9:1}, 1.0 – 9.5 ppm) of **A**, the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (233mM) at 75 °C, pH 7, 6 d, followed by incubation at 75 °C, pH 12, 5 h, then readjustment to pH 7; **B**, First spike and **C**, second spike, with material from the reaction of glyceraldehyde 3-phosphate (**93**, 70mM) and sodium chlorite (98mM) at 0 °C, pH 4, 0.5 h (adjusted to pH 7 before spiking) and **D**, Material from **28** cyanohydrin hydrolysis (spectrum **A**) spiked with commercial glyceric acid 3-phosphate (**44**).

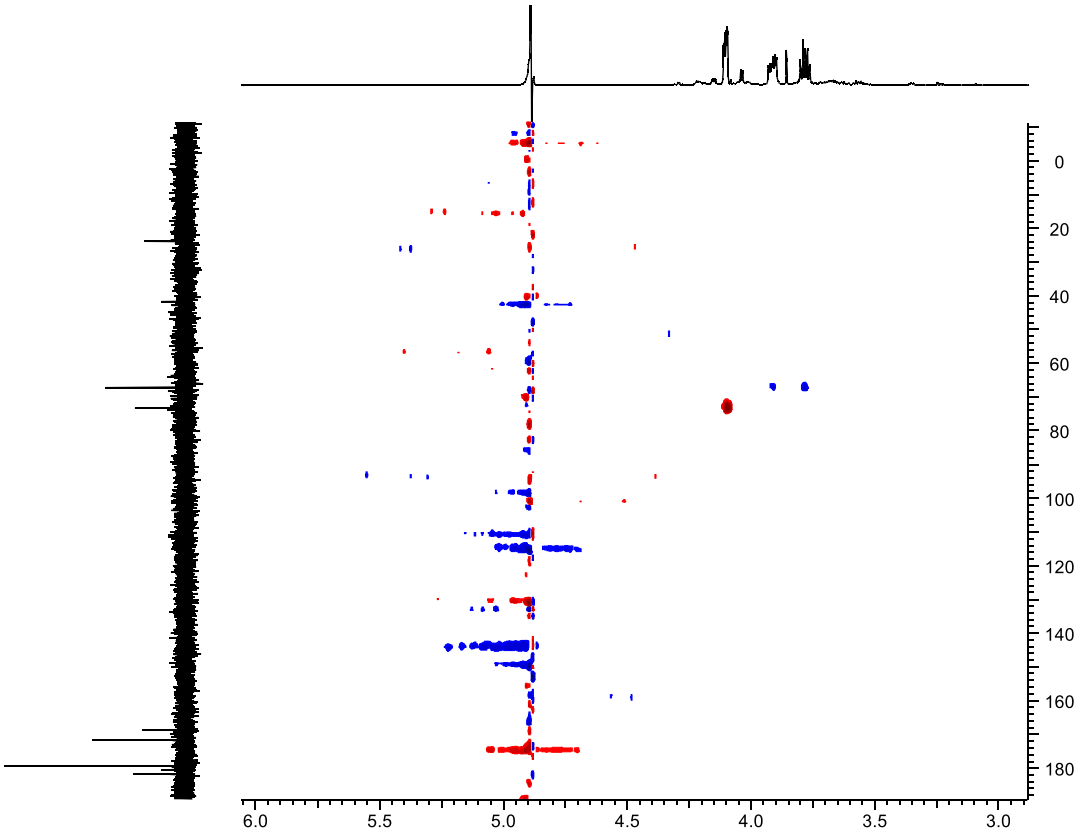




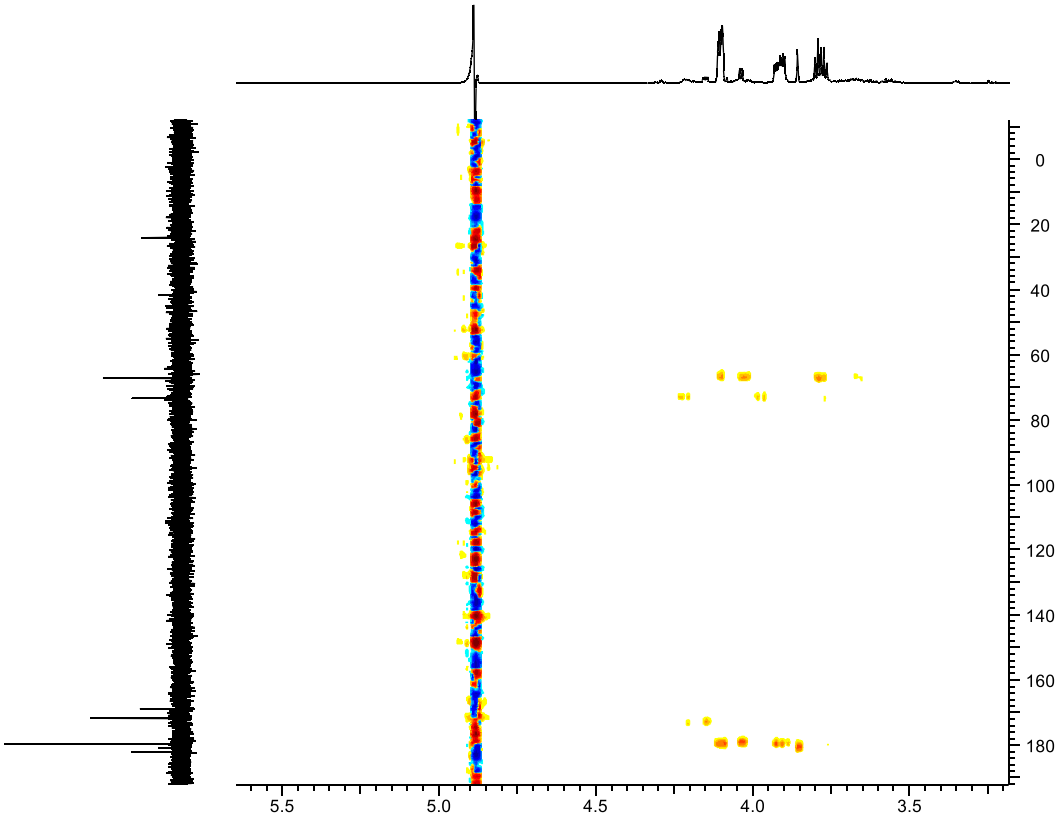
A117: ^{13}C NMR spectrum (151 MHz, {750mM phosphate, H_2O/D_2O, 9:1}, -20 – 20 ppm) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (233mM) at 75 °C, pH 7, 6 days, followed by incubation at 75 °C, pH 12, 5 h, with expanded peaks overlaid.



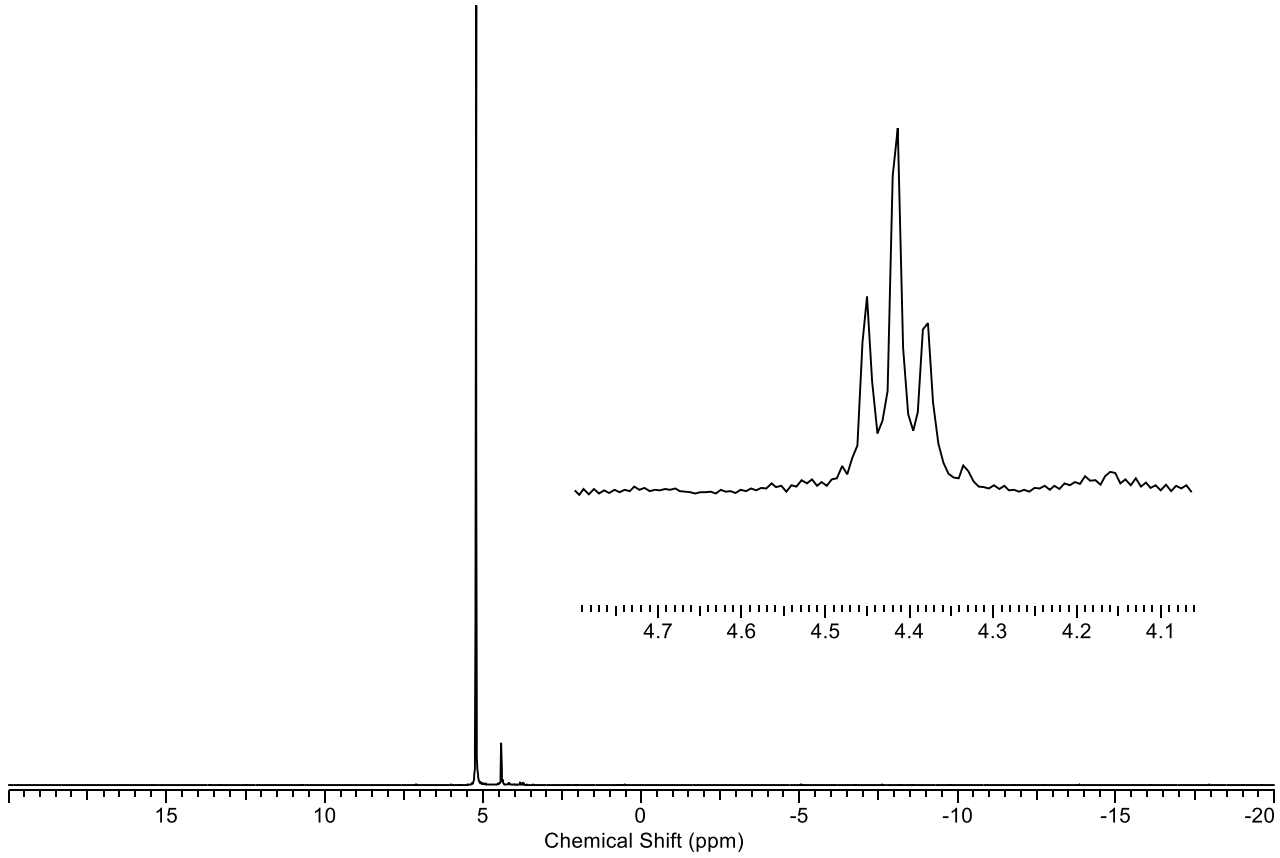
A118: ^{1}H-^{13}C HSQC NMR spectrum (600 MHz, {750mM phosphate, H_2O/D_2O, 9:1}) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (233mM) at 75 °C, pH 7, 6 days, followed by incubation at 75 °C, pH 12, 5 h.



A119: ^{1}H-^{13}C HMBC NMR spectrum (600 MHz, {750mM phosphate, H_2O/D_2O, 9:1}) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (233mM) at 75 °C, pH 7, 6 days, followed by incubation at 75 °C, pH 12, 5 h.

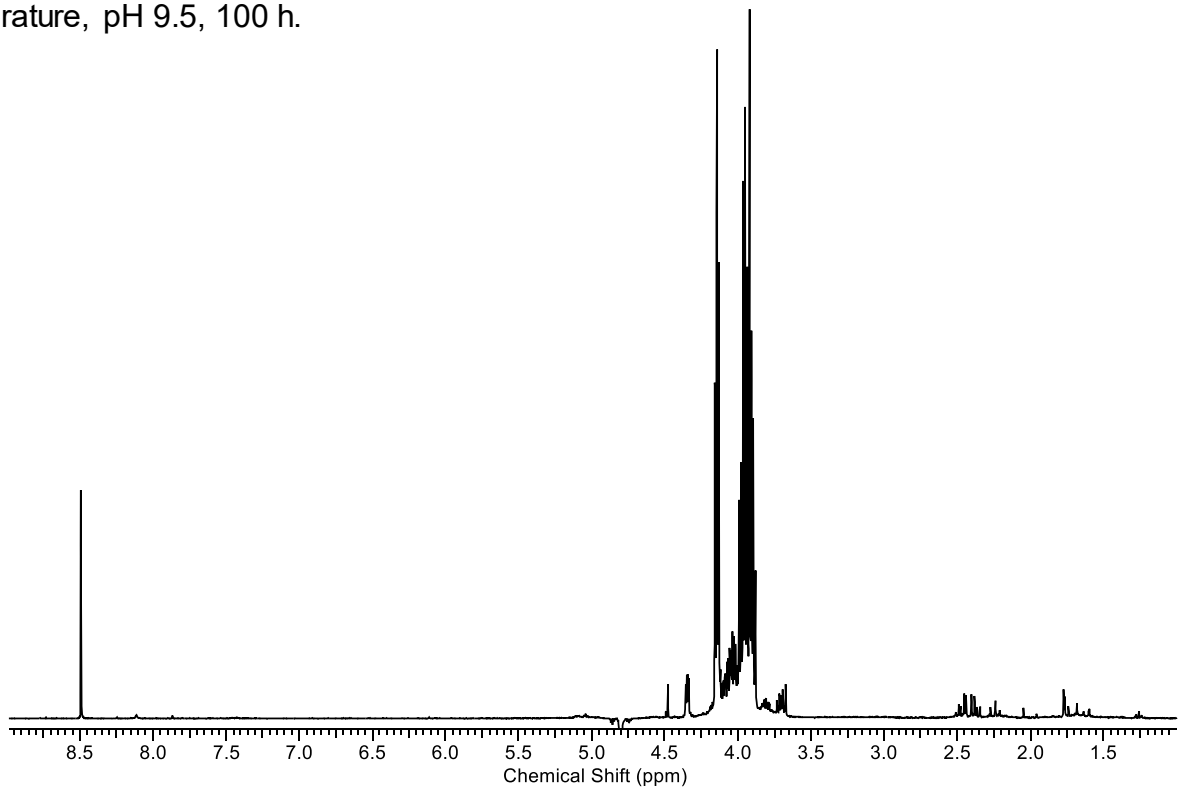


A120: ^{31}P NMR spectrum (161 MHz, {750mM phosphate, H_2O/D_2O, 9:1} -20 – 20 ppm) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (233mM) at 75 °C, pH 7, 6 days, followed by incubation at 75 °C, pH 12, 5 h, with expansion overlaid.

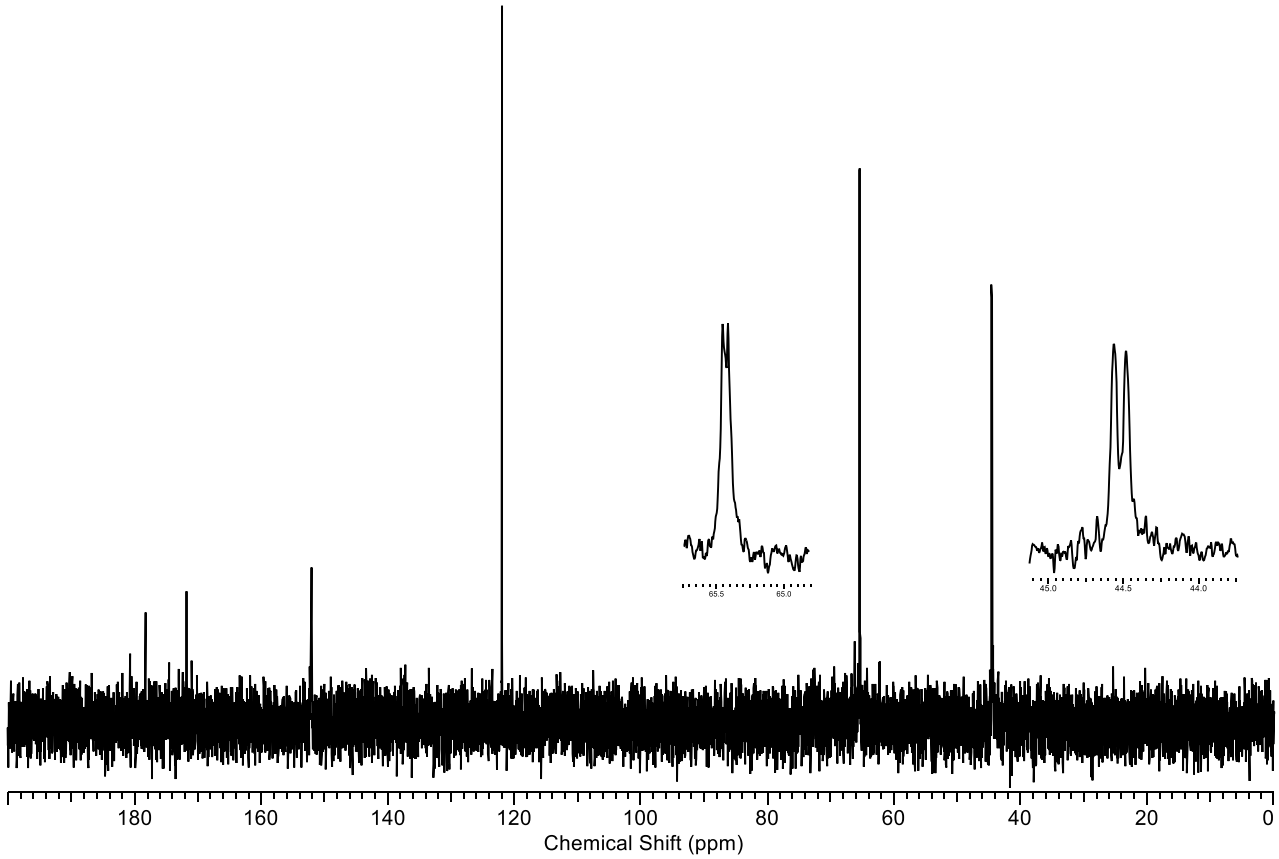


Glycolaldehyde phosphate aminonitrile (**128**)

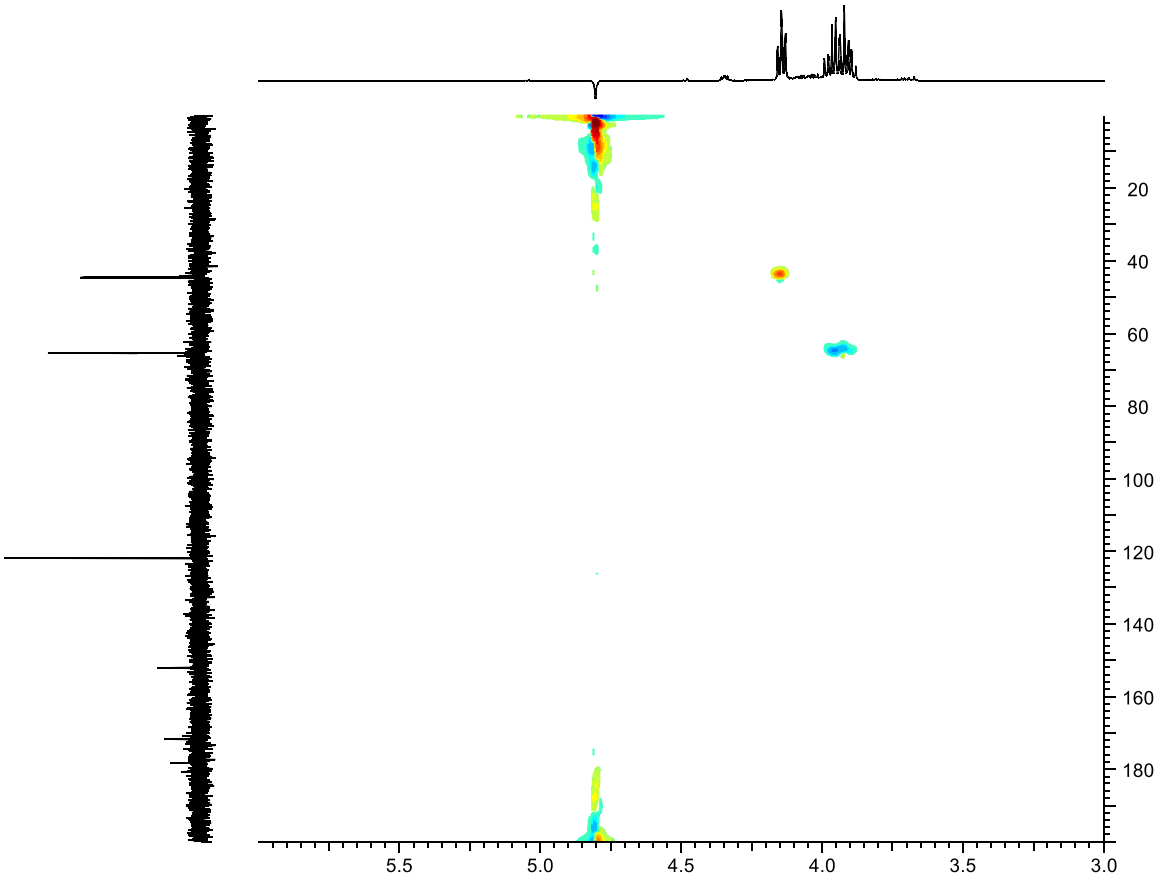
A121: ^{1}H NMR spectrum (400 MHz, {H_2O/D_2O, 9:1}, 1.0 – 9.0 ppm) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (600mM) and ammonium chloride (1M) at ambient temperature, pH 9.5, 100 h.



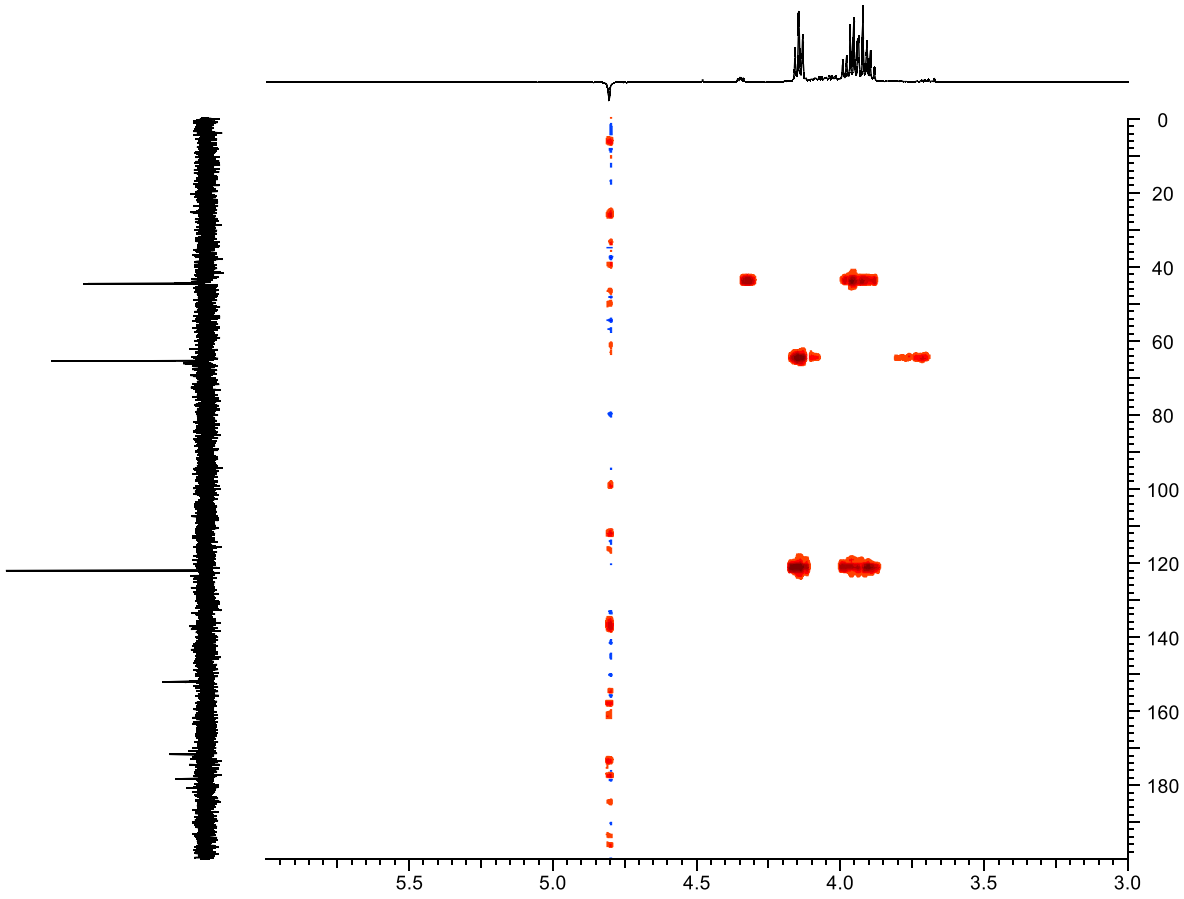
A122: ^{13}C NMR spectrum (100 MHz, {H_2O/D_2O, 9:1}, 0 – 200 ppm) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (600mM) and ammonium chloride (1M) at ambient temperature, pH 9.5, 100 h, with expanded peaks overlaid.



A123: ^{1}H-^{13}C HSQC NMR spectrum (400 MHz, {H_2O/D_2O, 9:1}) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (600mM) and ammonium chloride (1M) at ambient temperature, pH 9.5, 100 h.

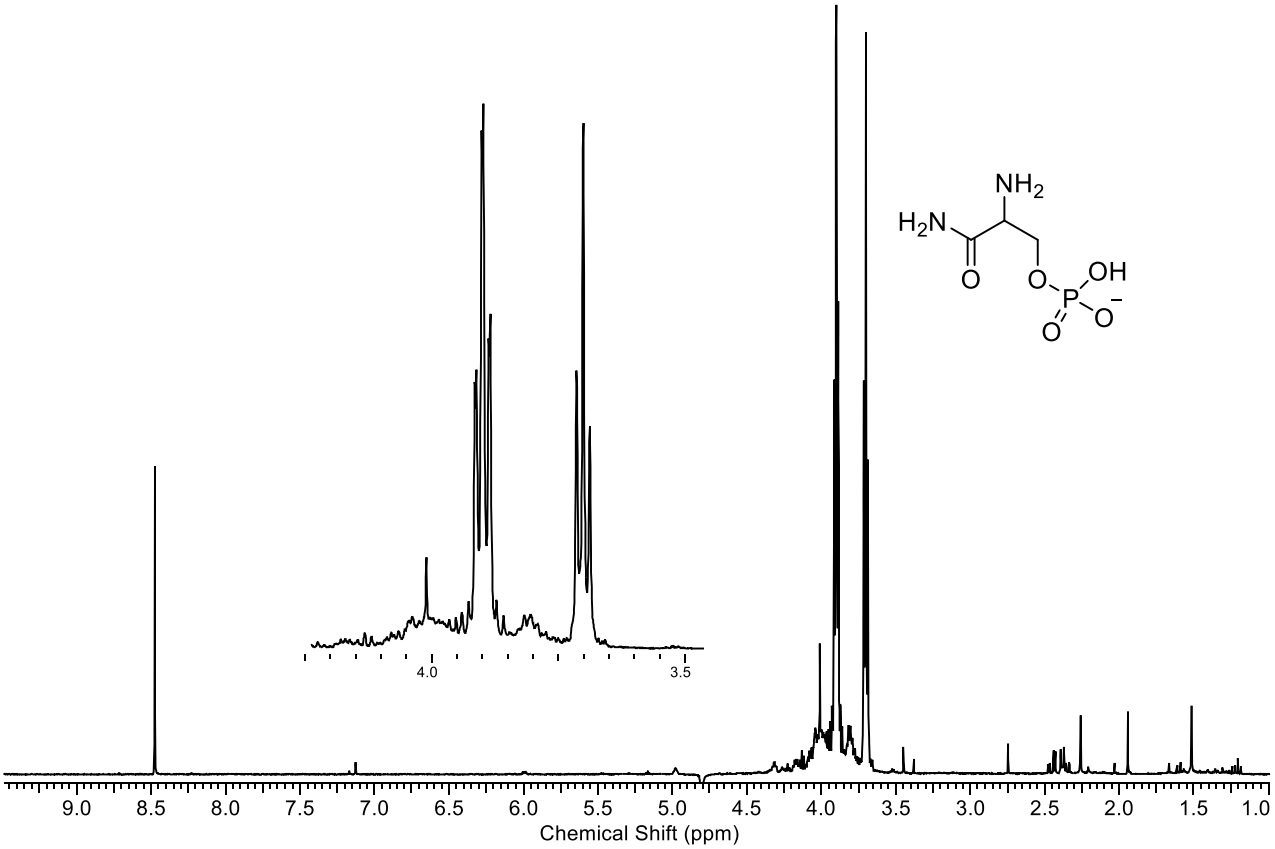


A124: ^{1}H-^{13}C HMBC NMR spectrum (400 MHz, {H_2O/D_2O, 9:1}) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (600mM) and ammonium chloride (1M) at ambient temperature, pH 9.5, 100 h.

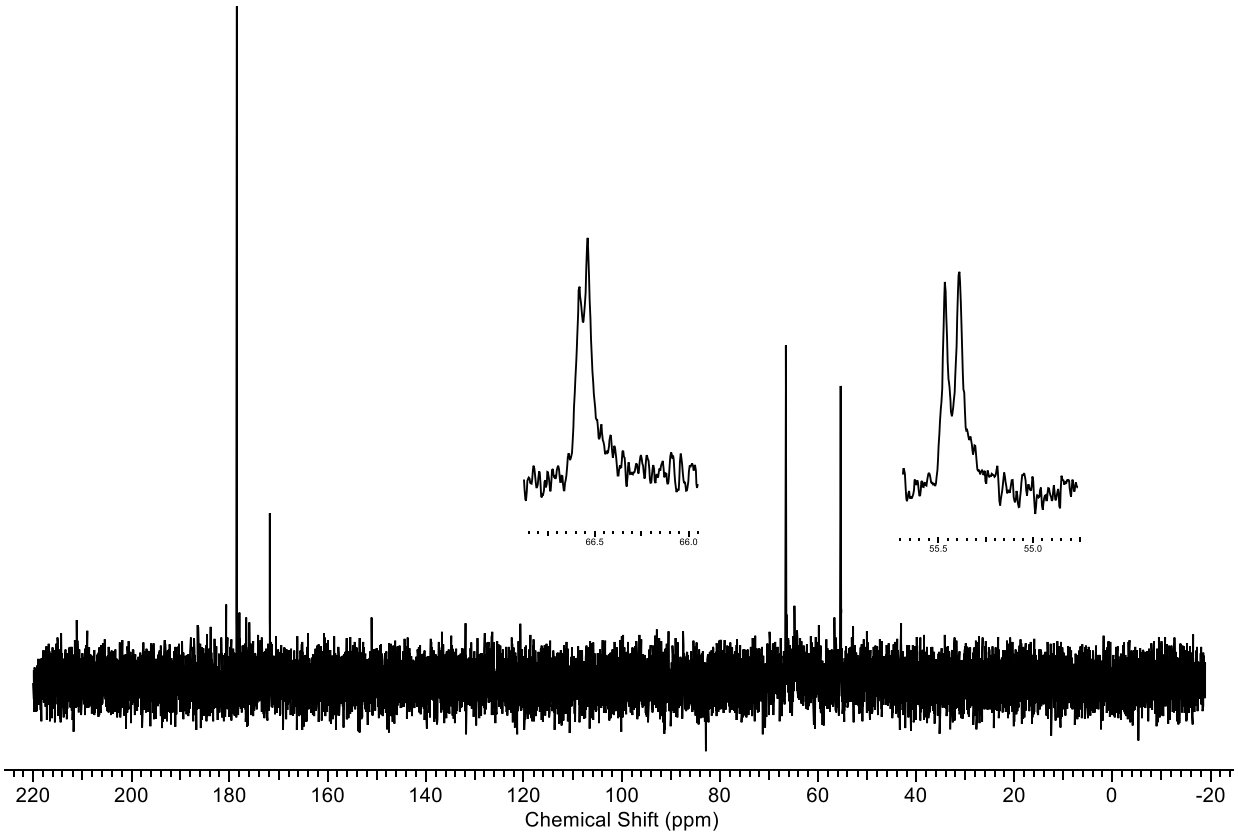


Serinamide-3-phosphate (**130**)

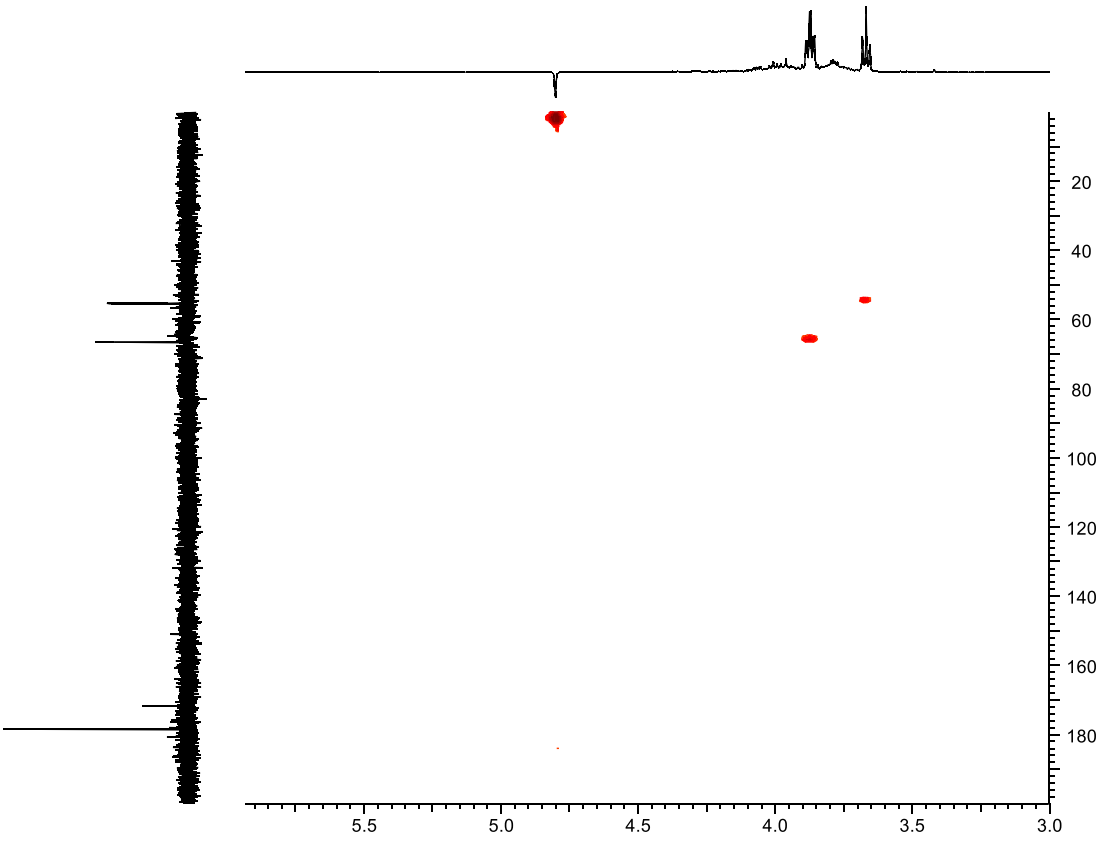
A125: ^{1}H NMR spectrum (600 MHz, {H_2O/D_2O, 9:1}, 1.0 – 9.5 ppm) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (180mM) and ammonium chloride (1M) at ambient temperature, pH 9.5, 3 days, with expanded peaks overlaid.



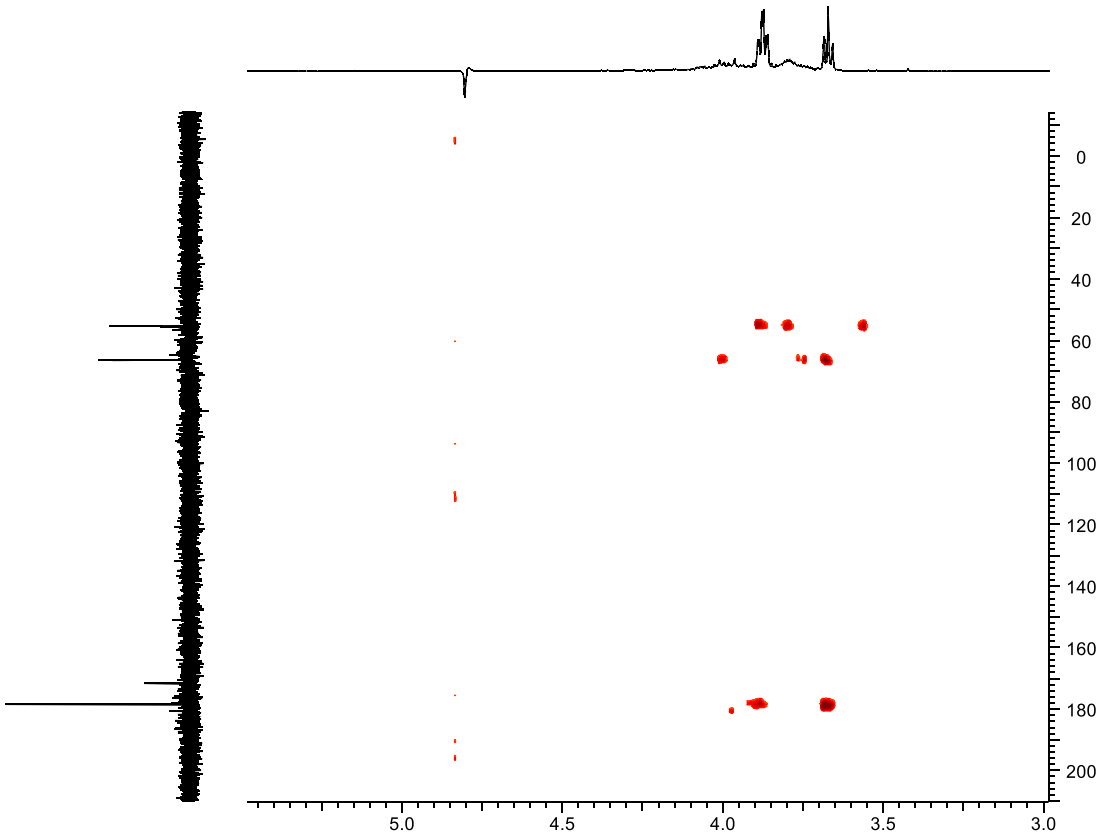
A126: ^{13}C NMR spectrum (100 MHz, {H_2O/D_2O, 9:1}, -20 – 220 ppm) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (180mM) and ammonium chloride (1M) at ambient temperature, pH 9.5, 3 days, with expanded peaks overlaid.



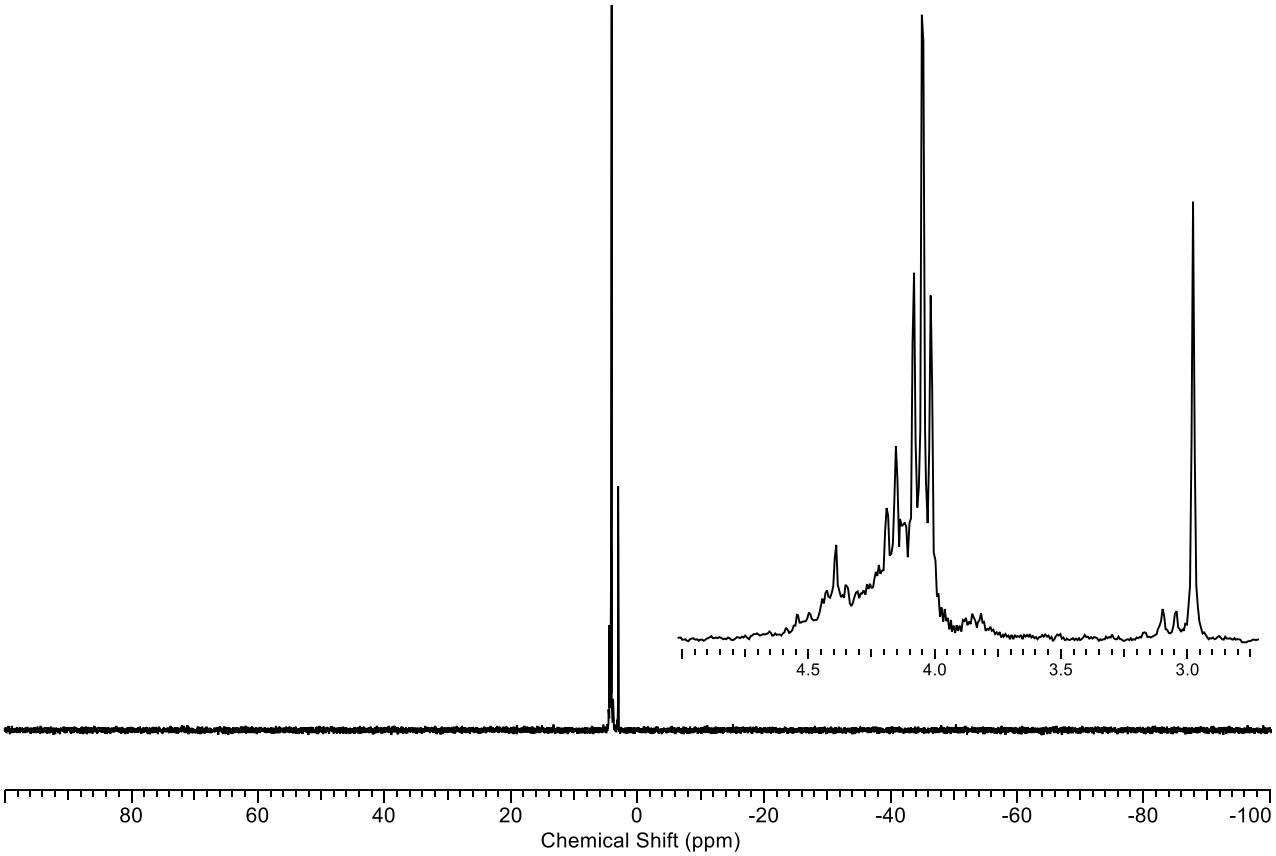
A127: ^{1}H-^{13}C HSQC NMR spectrum (400 MHz, {H_2O/D_2O, 9:1}) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (180mM) and ammonium chloride (1M) at ambient temperature, pH 9.5, 3 days.



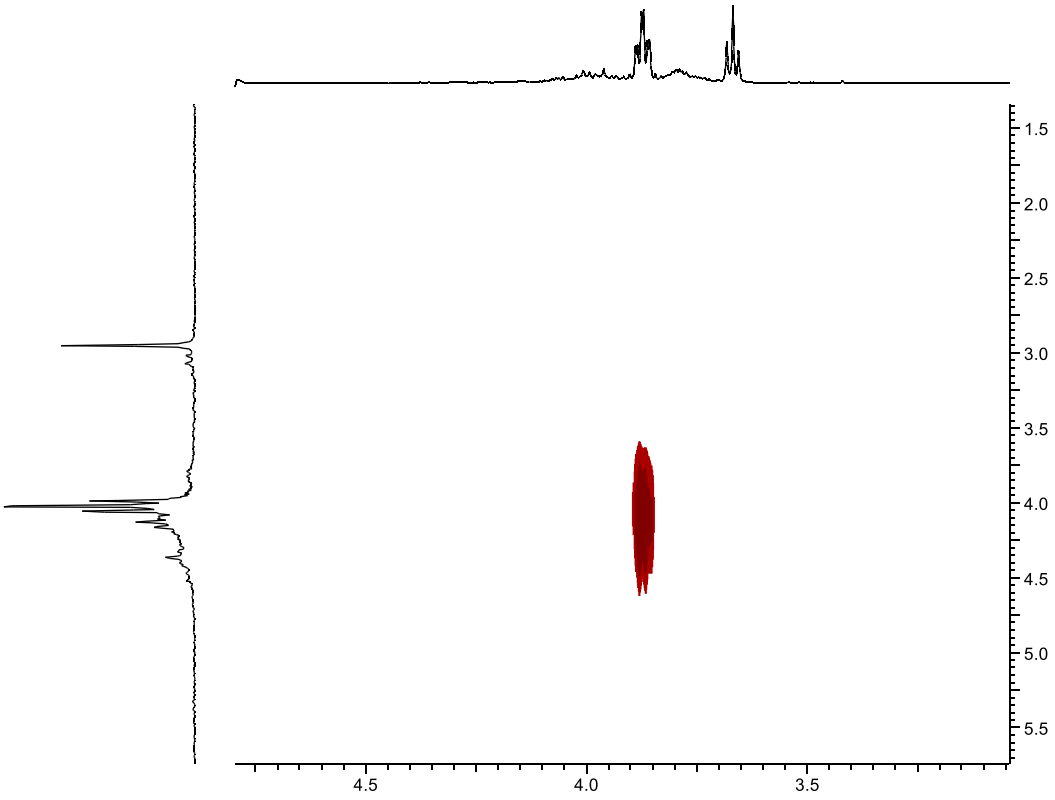
A128: ^{1}H-^{13}C HMBC NMR spectrum (400 MHz, {H_2O/D_2O, 9:1}) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (180mM) and ammonium chloride (1M) at ambient temperature, pH 9.5, 3 days.



A129: ^{31}P NMR spectrum (161 MHz, {750mM phosphate, H_2O/D_2O, 9:1} -20 – 20 ppm) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (180mM) and ammonium chloride (1M) at ambient temperature, pH 9.5, 3 days, with expansion ($^{31}P\{^1H$-coupled}) overlaid.

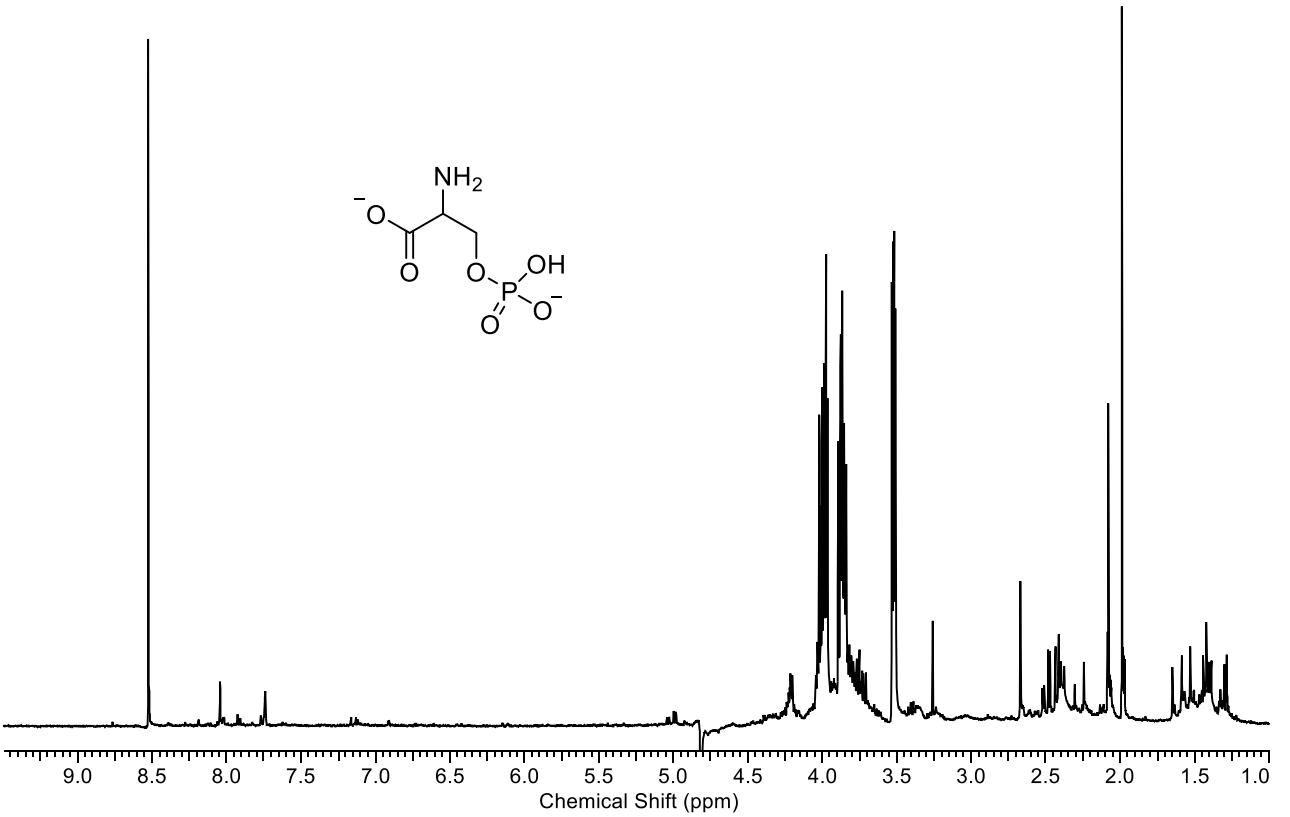


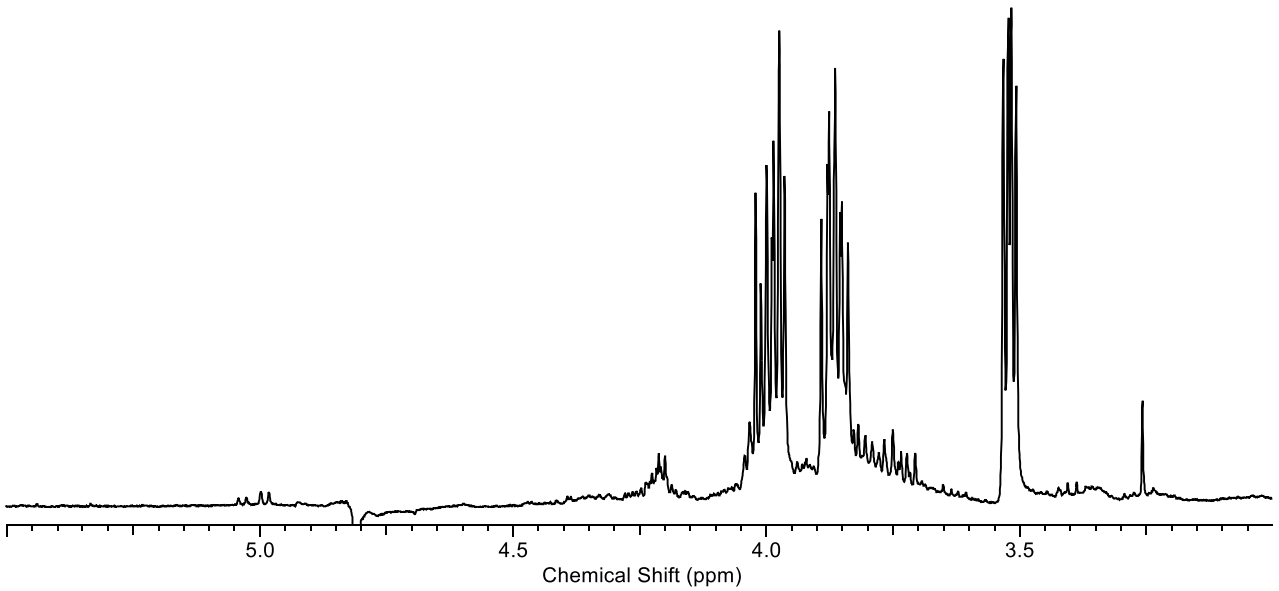
A130: ^{1}H-^{31}P HMBC NMR spectrum (400 MHz, {H_2O/D_2O, 9:1}) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (180mM) and ammonium chloride (1M) at ambient temperature, pH 9.5, 3 days.



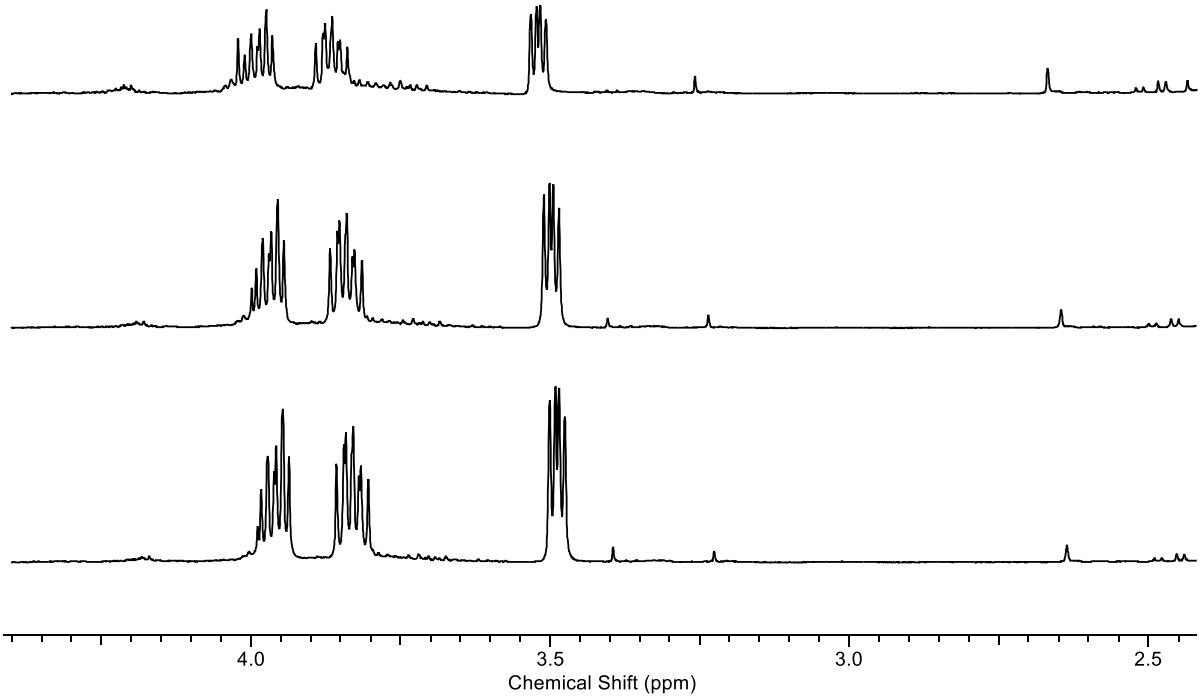
Serine-3-phosphate (**132**)

A131: ^{1}H NMR spectrum (600 MHz, {H_2O/D_2O, 9:1}, 1.0 – 9.5 ppm) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (150mM) and ammonium chloride (1M) after incubation at ambient temperature, pH 9.5, 100 h, then adjustment to pH 12 and incubation at 75 °C, 30 h (readjusted to pH 7 prior to analysis) with expansion below.

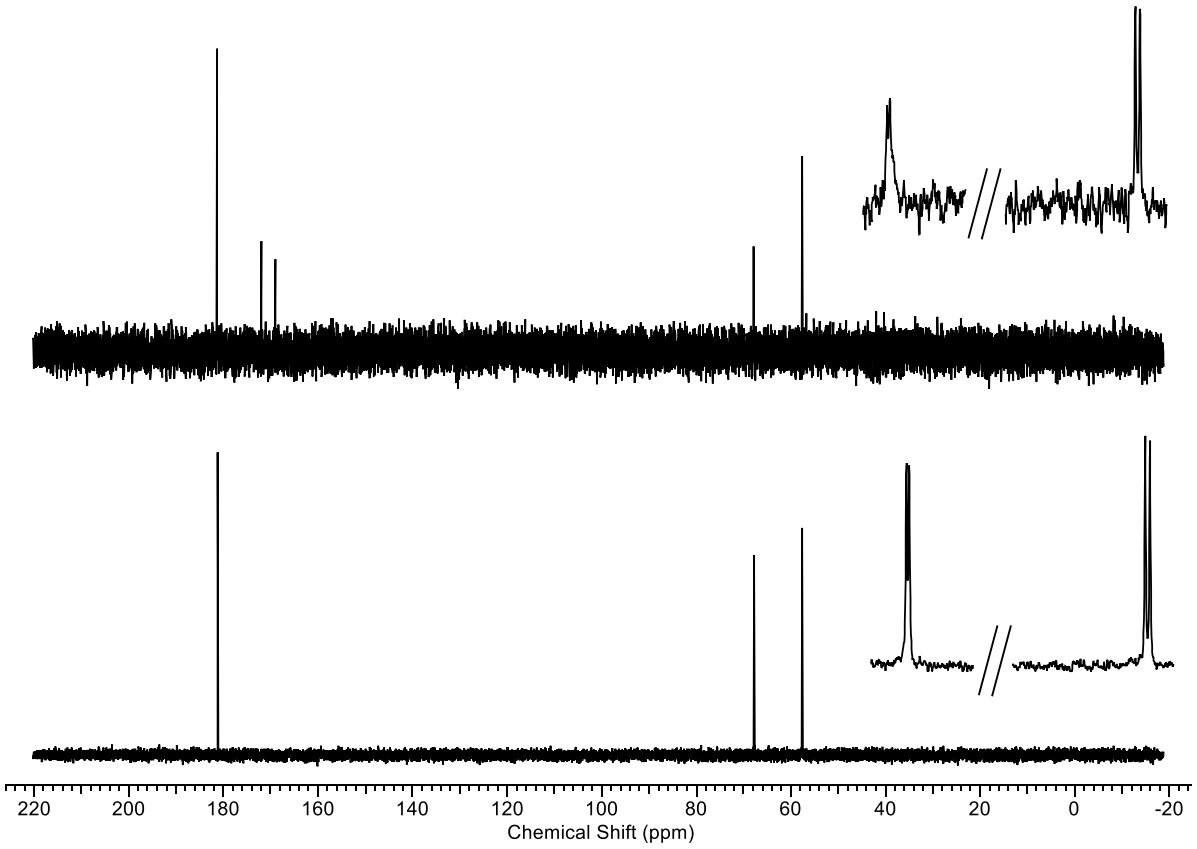




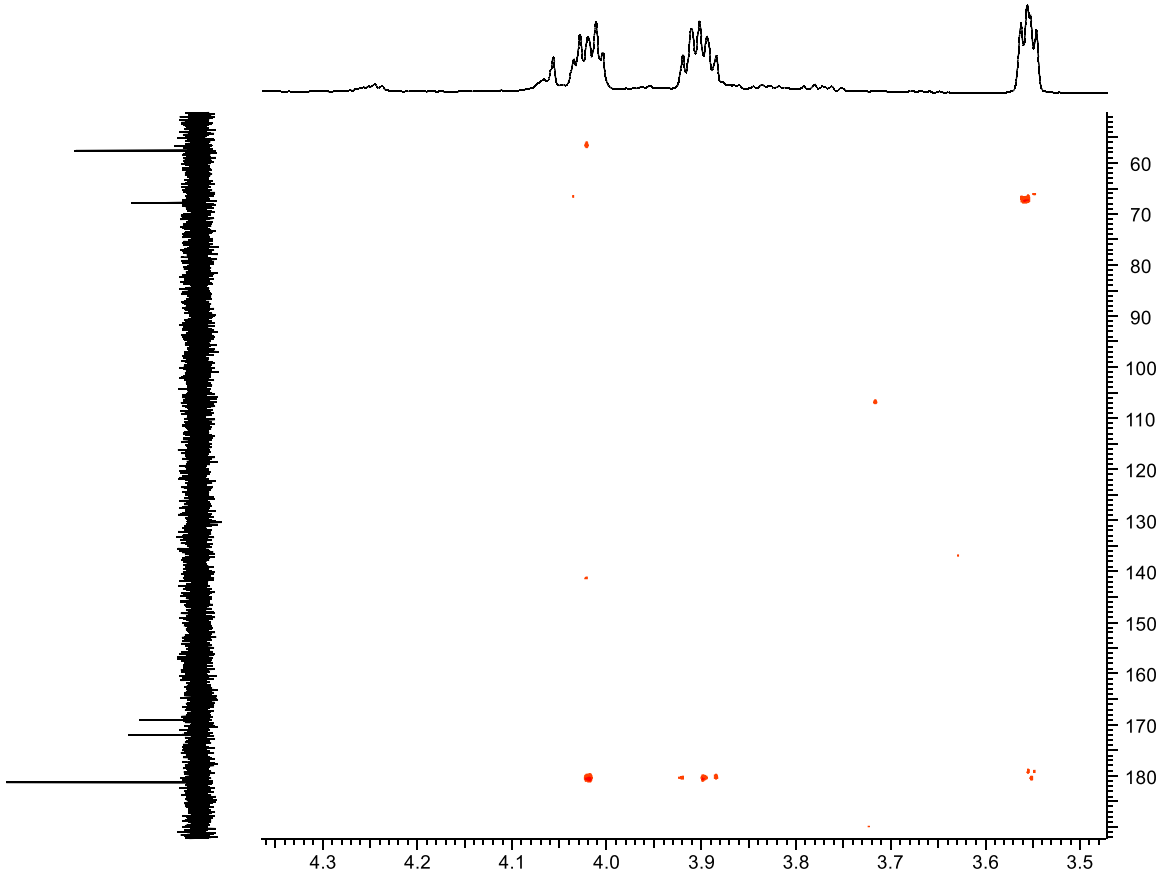
A132: ^{1}H NMR spectra (400 MHz, {H_2O/D_2O, 9:1}, 2.4 – 4.4 ppm) of **Top**, the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (150mM) and ammonium chloride (1M) after incubation at ambient temperature, pH 9.5, 100 h, then adjustment to pH 12 and incubation at 75 °C, 30 h (readjusted to pH 7 prior to analysis); **Middle**, first spike and **Bottom**, second spike, with commercial phosphoserine (**132**).



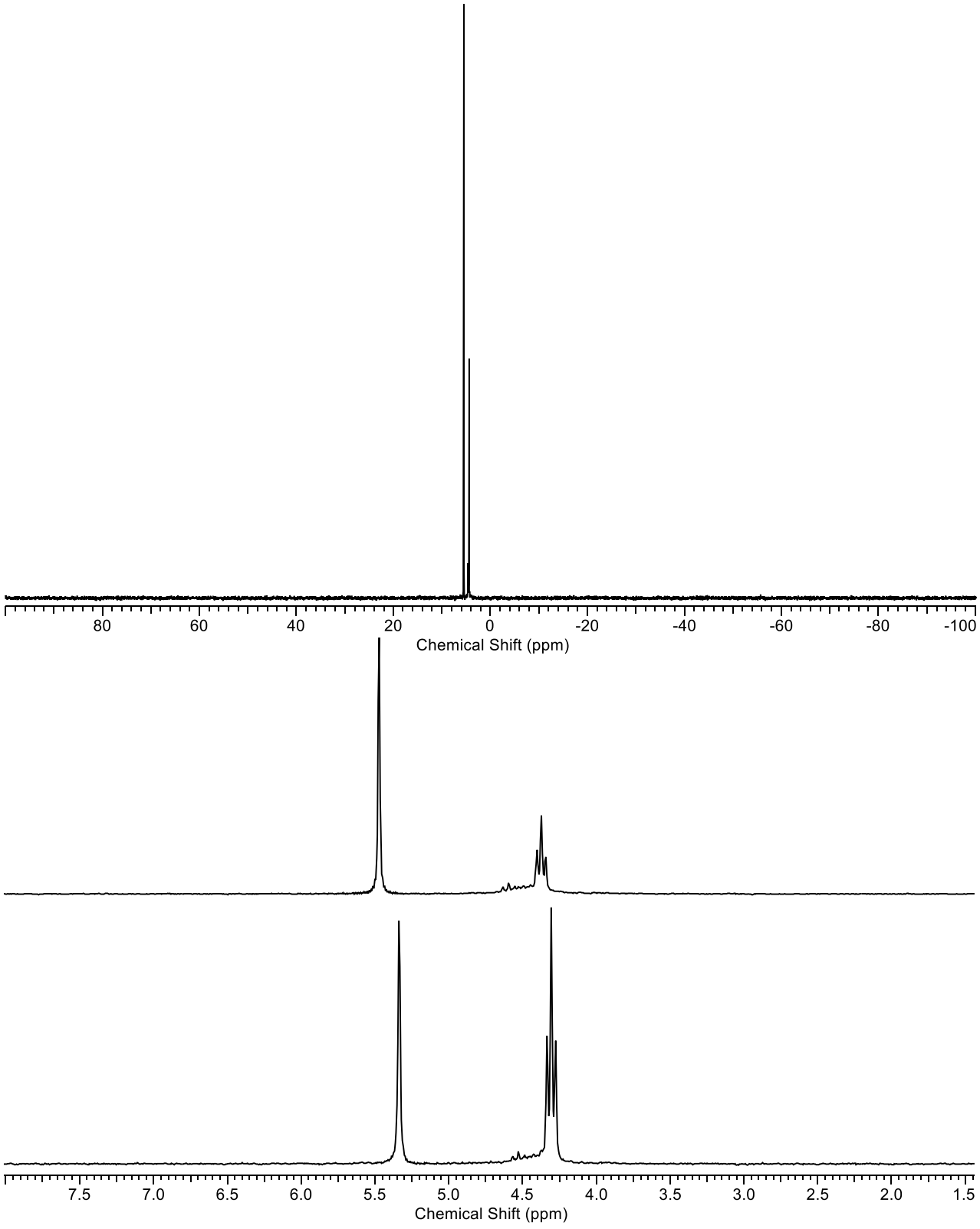
A133: ^{13}C NMR spectra (151 MHz, {H_2O/D_2O, 9:1}, -20 – 220 ppm) of **Top**, the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (150mM) and ammonium chloride (1M) after incubation at ambient temperature, pH 9.5, 100 h, then adjustment to pH 12 and incubation at 75 °C, 30 h (readjusted to pH 7 prior to analysis) and **Bottom**, commercial phosphoserine (**132**), with expanded signals overlaid.



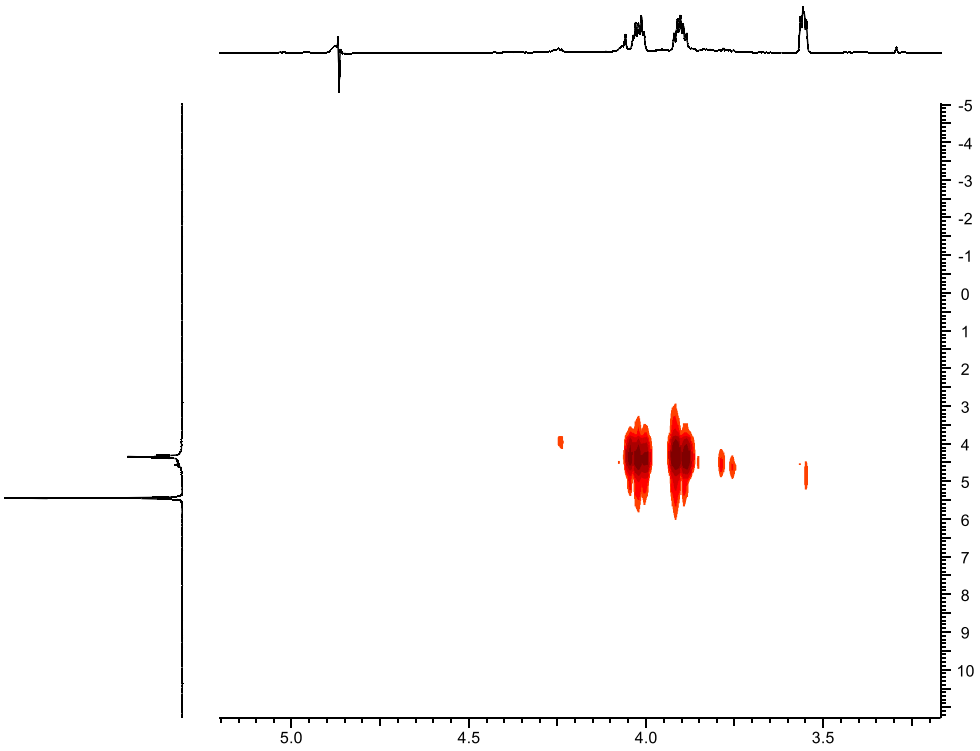
A134: ^{1}H-^{13}C HMBC NMR spectrum (600 MHz, {H_2O/D_2O, 9:1}) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (150mM) and ammonium chloride (1M) after incubation at ambient temperature, pH 9.5, 100 h, then adjustment to pH 12 and incubation at 75 °C, 30 h (readjusted to pH 7 prior to analysis).



A135: ^{31}P NMR spectrum (161 MHz, {H_2O/D_2O, 9:1} -100 – 100 ppm) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (150mM) and ammonium chloride (1M) after incubation at ambient temperature, pH 9.5, 100 h, then adjustment to pH 12 and incubation at 75 °C, 30 h (readjusted to pH 7 prior to analysis) with an expansion of the ^{1}H-coupled spectrum and subsequent spike with commercial phosphoserine (**132**), below.

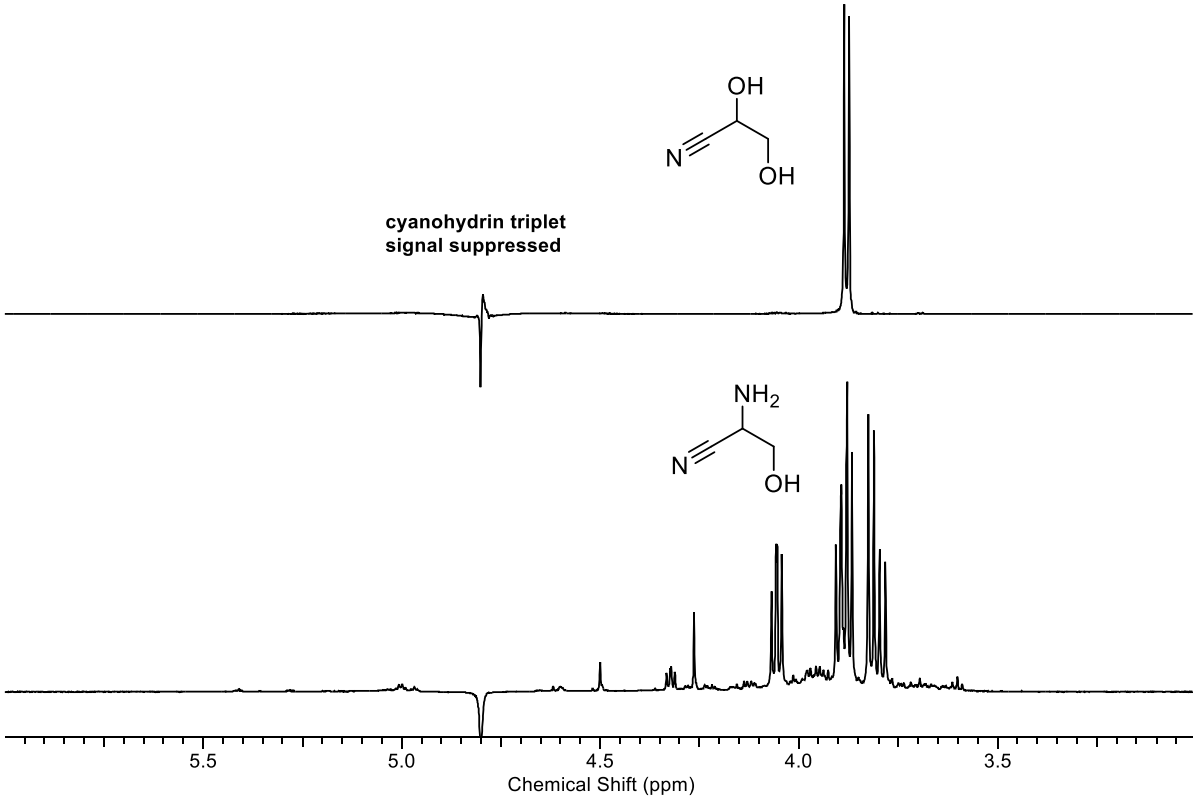


A136: ^{1}H-^{31}P HMBC NMR spectrum (400 MHz, {H_2O/D_2O, 9:1}) of the reaction of glycolaldehyde phosphate (**28**, 200mM) and sodium cyanide (150mM) and ammonium chloride (1M) after incubation at ambient temperature, pH 9.5, 100 h, then adjustment to pH 12 and incubation at 75 °C, 30 h (readjusted to pH 7 prior to analysis).



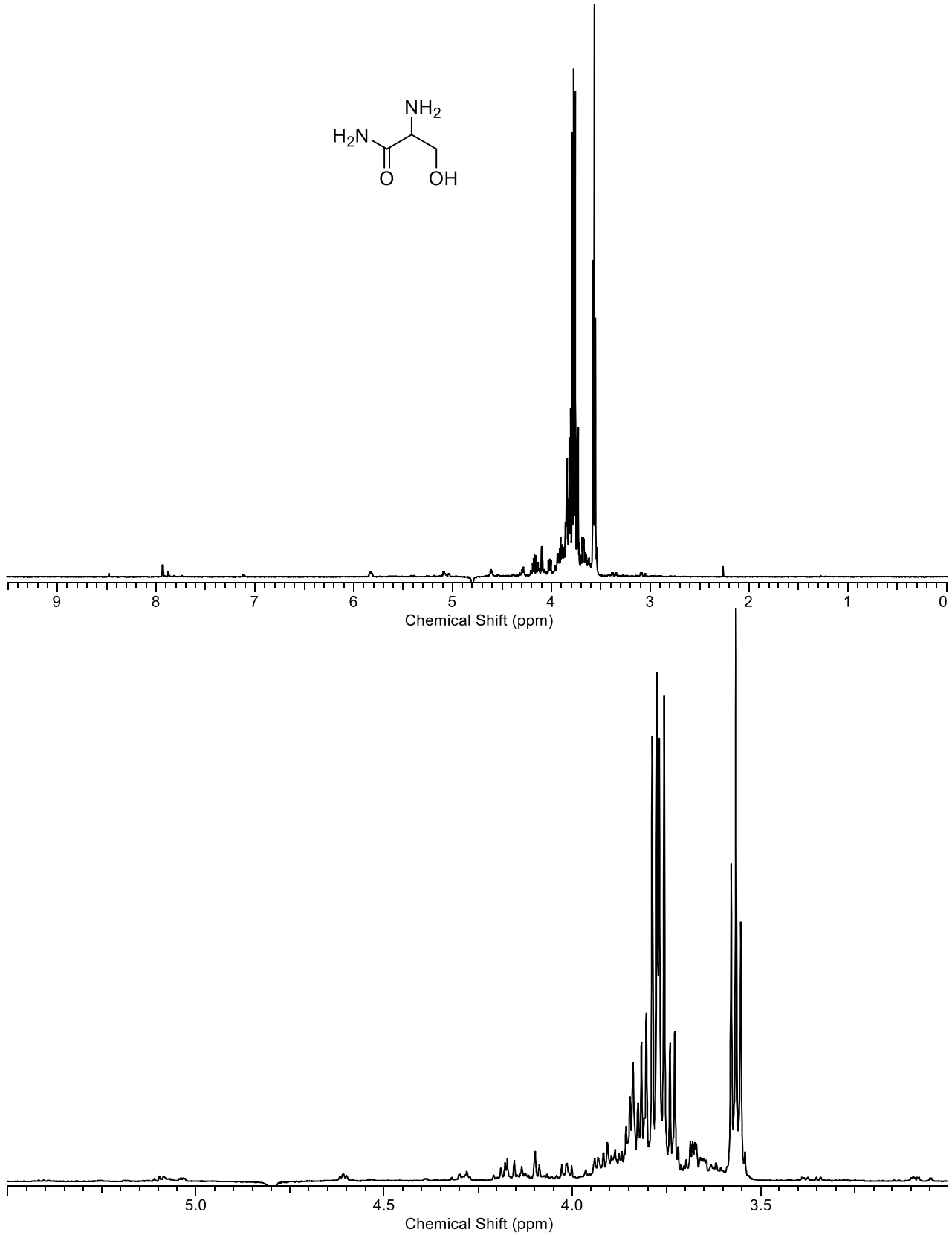
Glycolaldehyde aminonitrile (**86**)

A137: ^{1}H NMR spectra (400 MHz, {H_2O/D_2O, 9:1}, 3.0 – 6.0 ppm) of **Top**, glycolaldehyde (**26**, 200mM), sodium cyanide (600mM) and **Bottom**, after incubation with ammonium chloride (1M) at ambient temperature, pH 9.5 for 100 h.

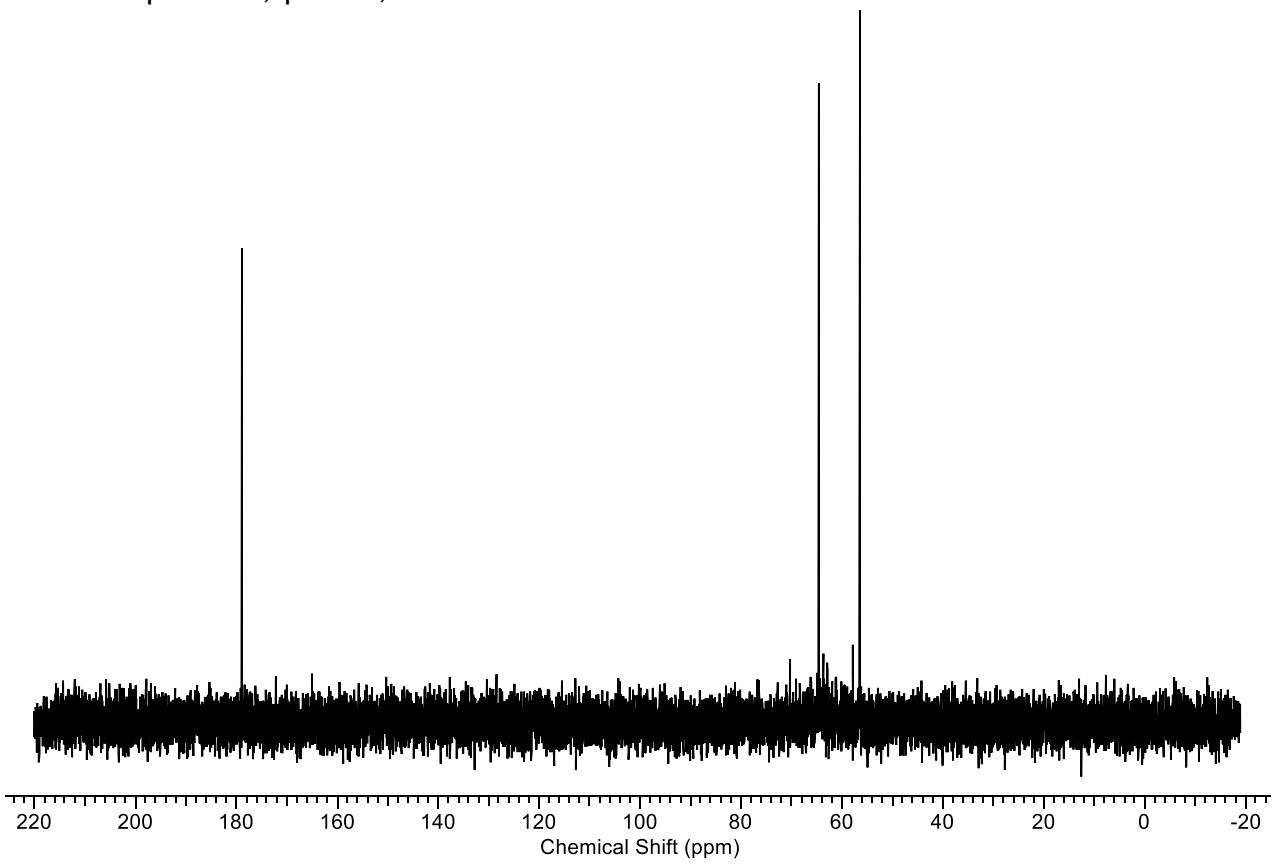


Serinamide (**131**)

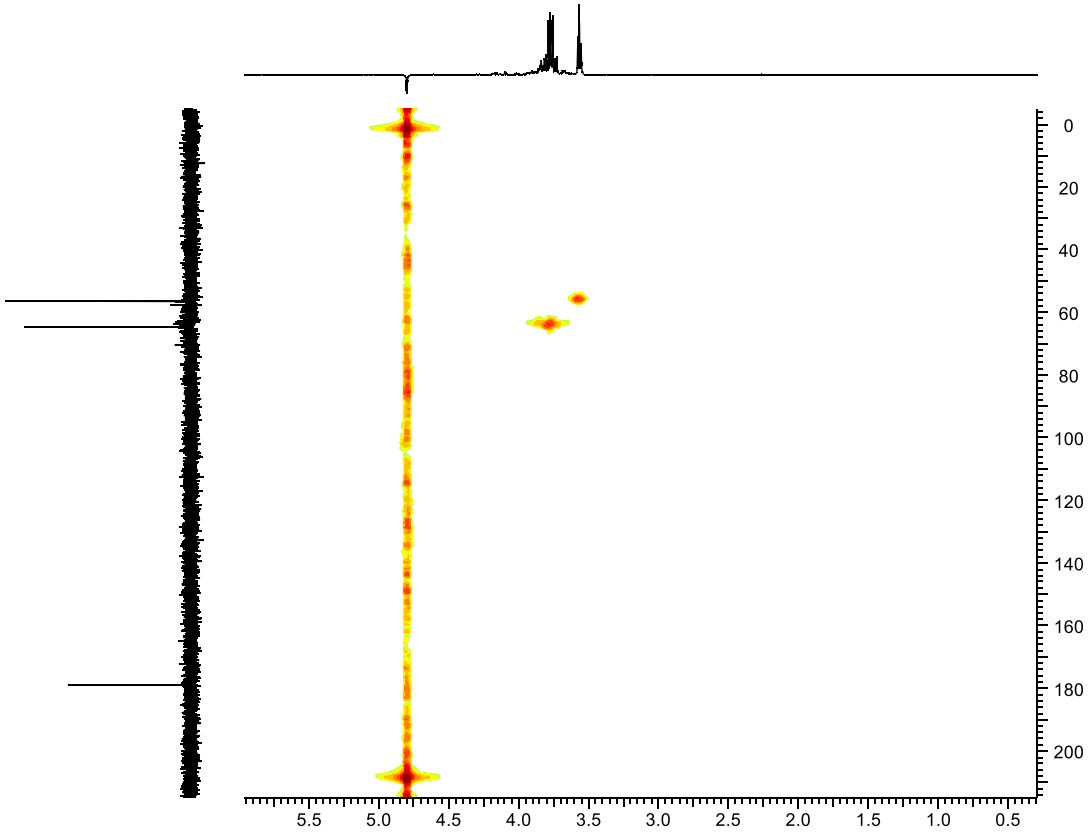
A138: ^{1}H NMR spectrum (600 MHz, {H_2O/D_2O, 9:1}, 0.0 – 9.5 ppm) of the reaction of glycolaldehyde (**26**, 200mM), sodium cyanide (150mM) and ammonium chloride (1M) after incubation at ambient temperature, pH 9.5, 100 h, with expansion below.



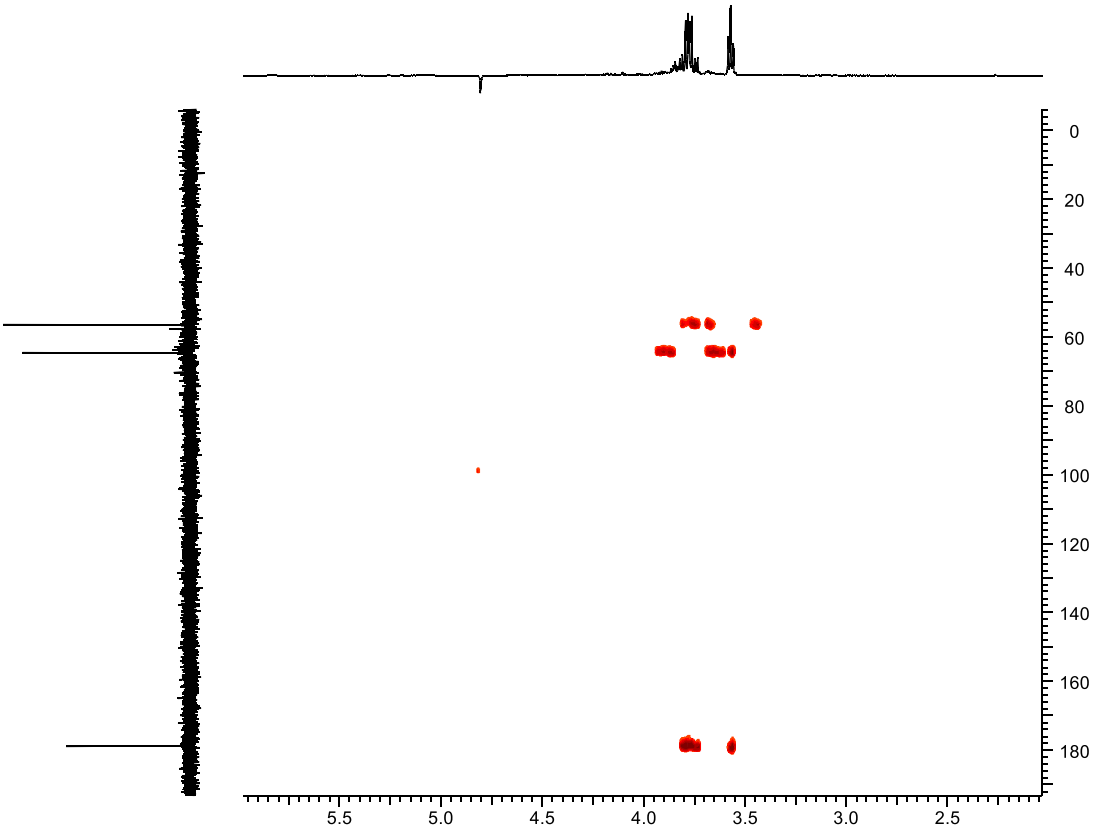
A139: ^{13}C NMR spectrum (151 MHz, {H_2O/D_2O, 9:1}, -20 – 220 ppm) of the reaction of glycolaldehyde (**26**, 200mM), sodium cyanide (150mM) and ammonium chloride (1M) after incubation at ambient temperature, pH 9.5, 100 h.



A140: ^{1}H-^{13}C HSQC NMR spectrum (600 MHz, {H_2O/D_2O, 9:1}) of glycolaldehyde (**26**, 200mM), sodium cyanide (150mM) and ammonium chloride (1M) after incubation at ambient temperature, pH 9.5, 100 h.

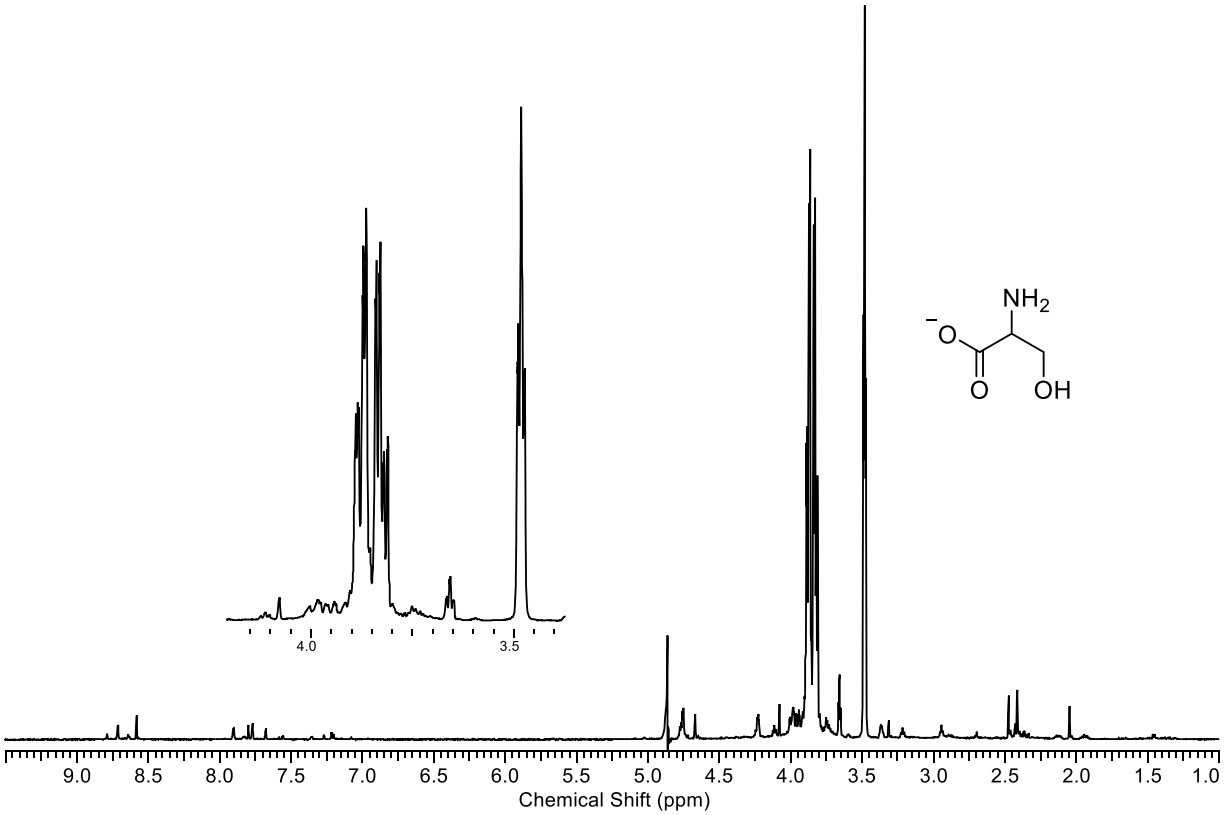


A141: ^{1}H-^{13}C HMBC NMR spectrum (600 MHz, {H_2O/D_2O, 9:1}) of glycolaldehyde (**26**, 200mM), sodium cyanide (150mM) and ammonium chloride (1M) after incubation at ambient temperature, pH 9.5, 100 h.

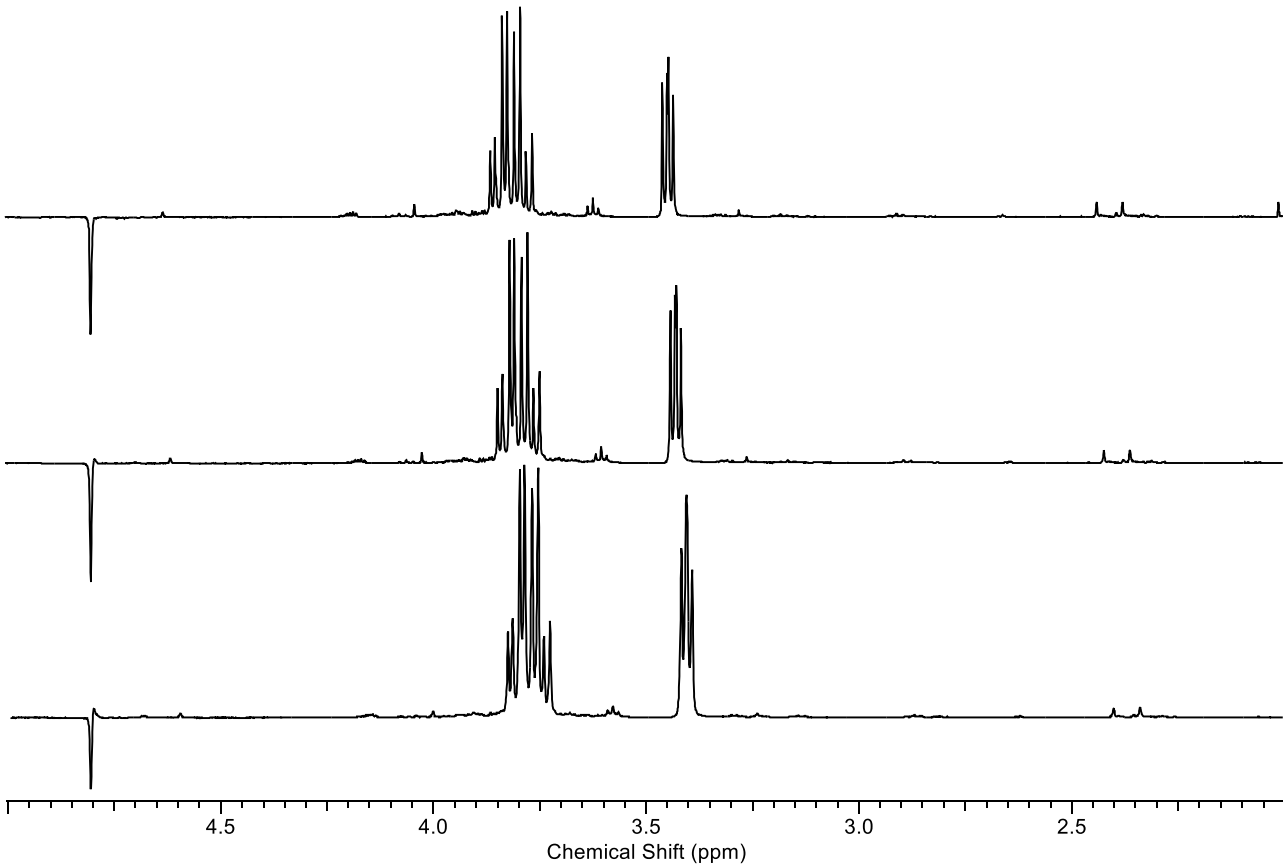


Serine (**129**)

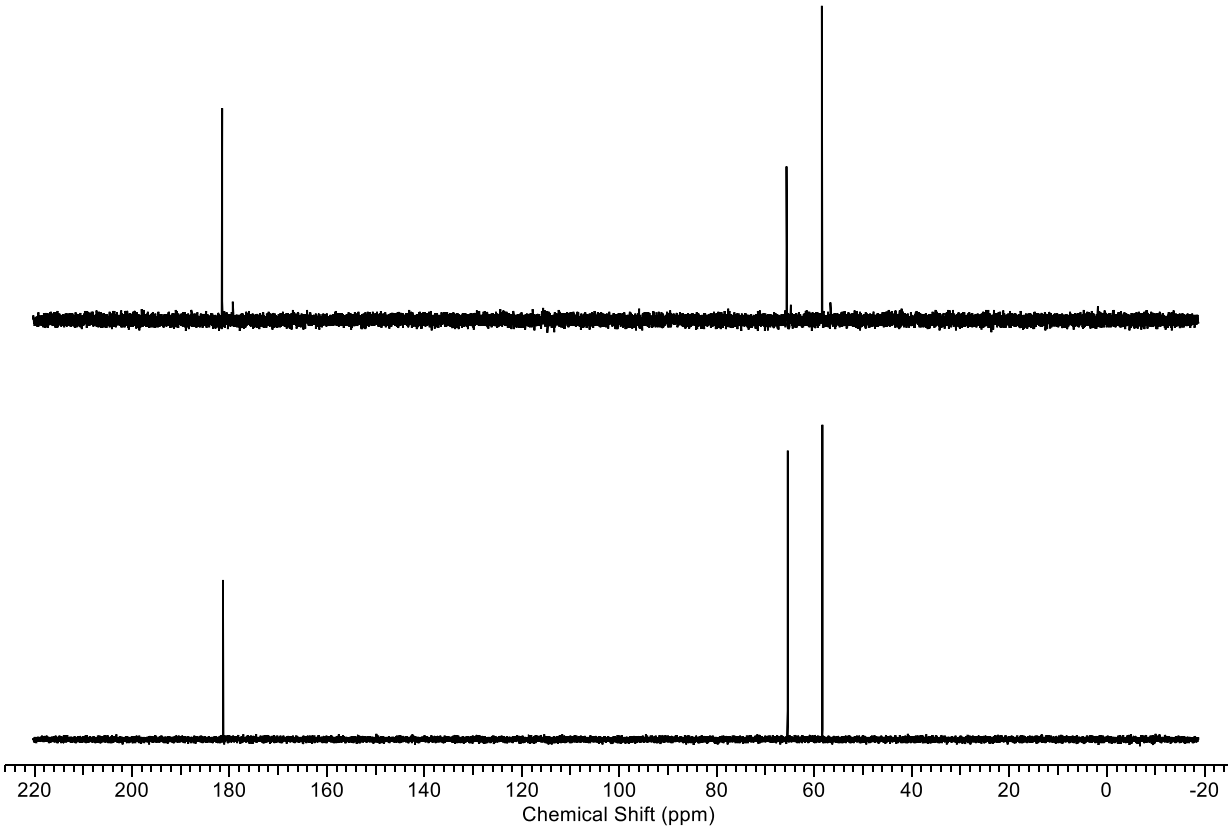
A142: ^{1}H NMR spectrum (600 MHz, {H_2O/D_2O, 9:1}, 1.0 – 9.5 ppm) of the reaction of glycolaldehyde (**26**, 200mM), sodium cyanide (150mM) and ammonium chloride (1M) after incubation at ambient temperature, pH 9.5, 100 h, then adjustment to pH 12 and incubation at 75 °C, 30 h (readjusted from pH 10 to pH 12 after 26 h) with expansion overlaid.



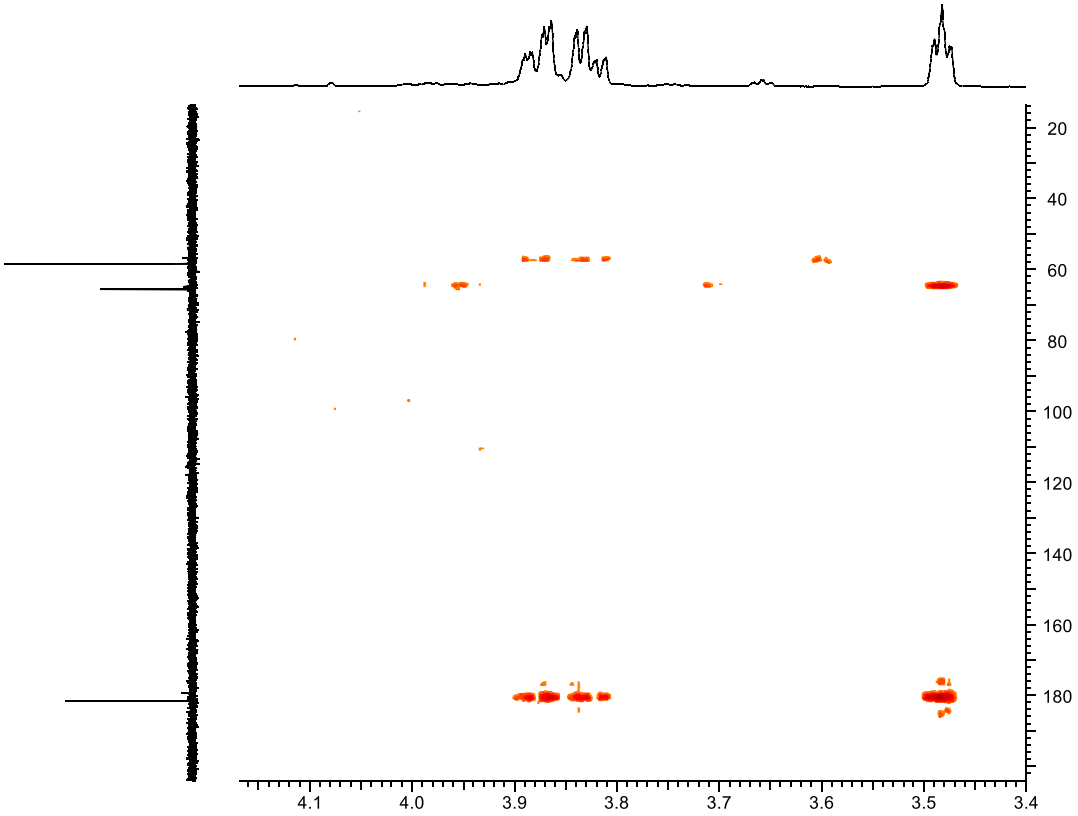
A143: ^{1}H NMR spectra (400 MHz, {H_2O/D_2O, 9:1}, 2.0 – 5.0 ppm) of **Top**, the reaction of glycolaldehyde (**26**, 200mM), sodium cyanide (150mM) and ammonium chloride (1M) after incubation at ambient temperature, pH 9.5, 100 h, then adjustment to pH 12 and incubation at 75 °C, 30 h (readjusted to pH 7 prior to analysis); **Middle**, first spike and **Bottom**, second spike, with commercial serine (**129**).



A144: ^{13}C NMR spectra (151 MHz, {H_2O/D_2O, 9:1}, -20 – 220 ppm) of **Top**, the reaction of glycolaldehyde (**26**, 200mM), sodium cyanide (150mM) and ammonium chloride (1M) after incubation at ambient temperature, pH 9.5, 100 h, then adjustment to pH 12 and incubation at 75 °C, 30 h and **Bottom**, commercial serine (**129**).

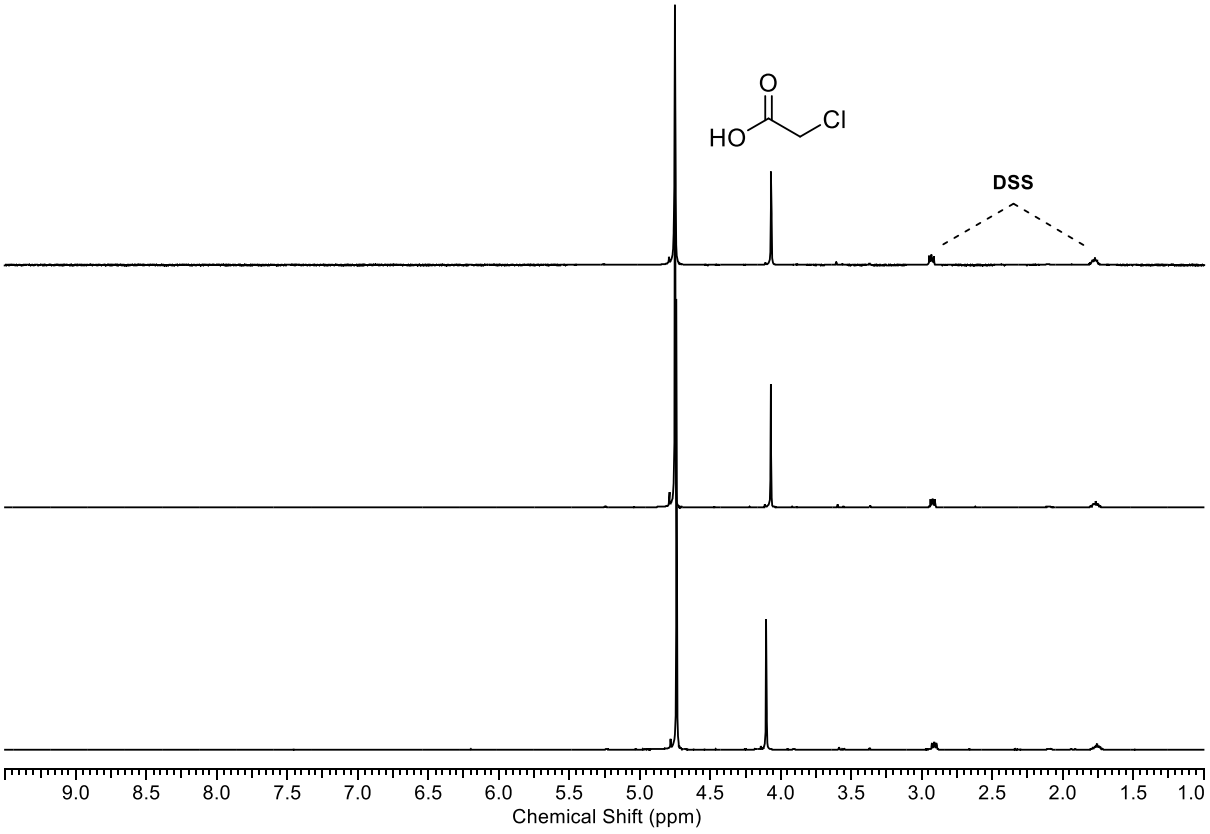


A145: ^{1}H-^{13}C HMBC NMR spectrum (600 MHz, {H_2O/D_2O, 9:1}) of the reaction of glycolaldehyde (**46**, 200mM), sodium cyanide (150mM) and ammonium chloride (1M) after incubation at ambient temperature, pH 9.5, 100 h, then adjustment to pH 12 and incubation at 75 °C, 30 h.



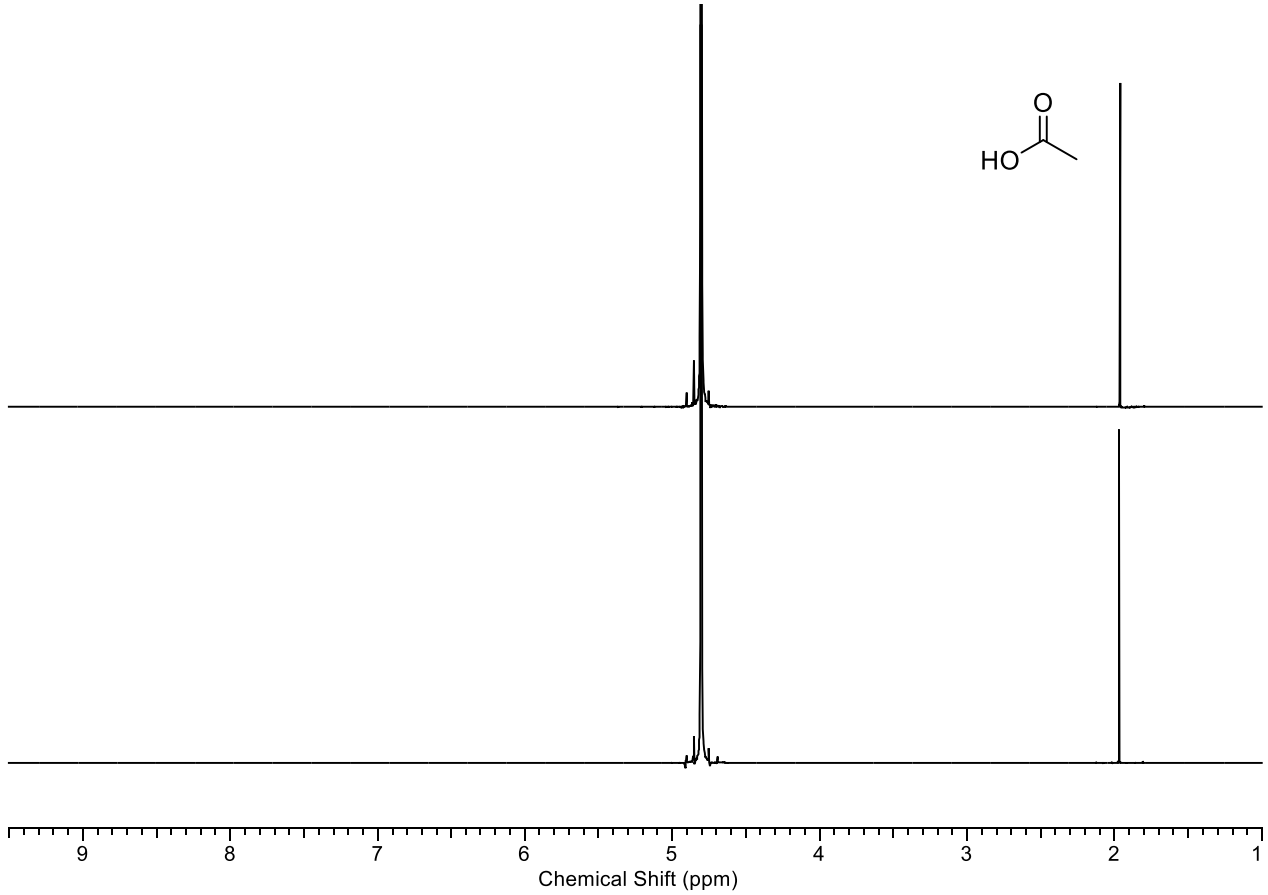
Chloroacetic acid (**123**)

A146: ^{1}H NMR spectra (400 MHz, {H_2O/D_2O, 9:1}, 1.0 – 9.5 ppm) of **Top**, the products of reaction of phosphoenolpyruvaldehyde (**105**, 70mM) and sodium chlorite (140mM) at 0 °C, pH 4, 2 h, with DSS after lyophilisation; **Middle**, after spiking with material from the reaction of phosphoenol pyruvate (**94**, 69mM) and sodium hypochlorite (140mM), 0 °C, pH 4, 1 h and **Bottom**, after spiking with commercial chloroacetic acid (**123**).



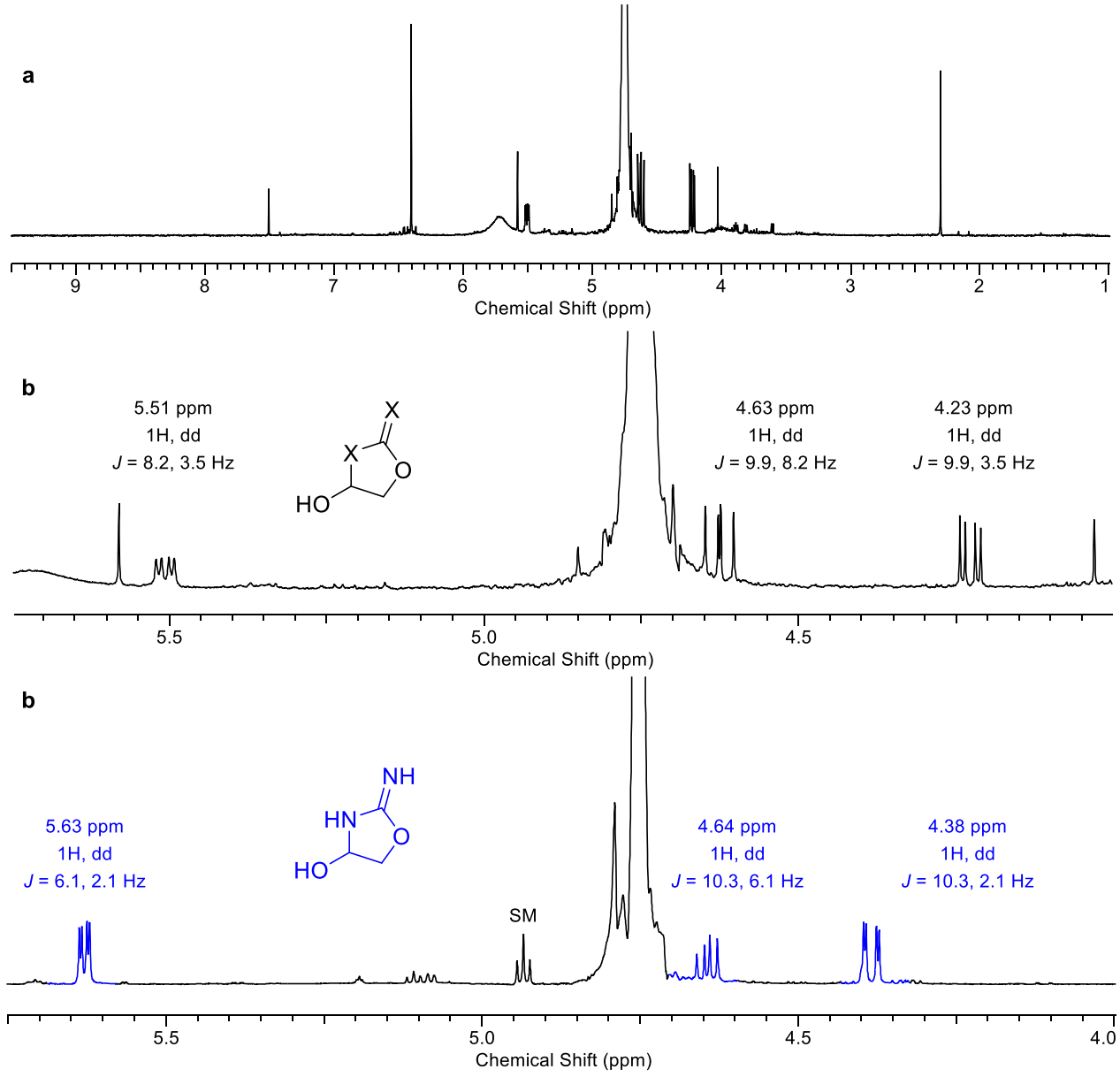
Acetic acid (**63**)

A147: ^{1}H NMR spectra (400 MHz, {H_2O/D_2O, 9:1}, 1.0 – 9.5 ppm) of **Top**, the reaction of sodium pyruvate (**64**, 69mM) and sodium chlorite (138mM) at ambient temperature, pH 4 and **Bottom**, after spiking with commercial acetate (**63**).

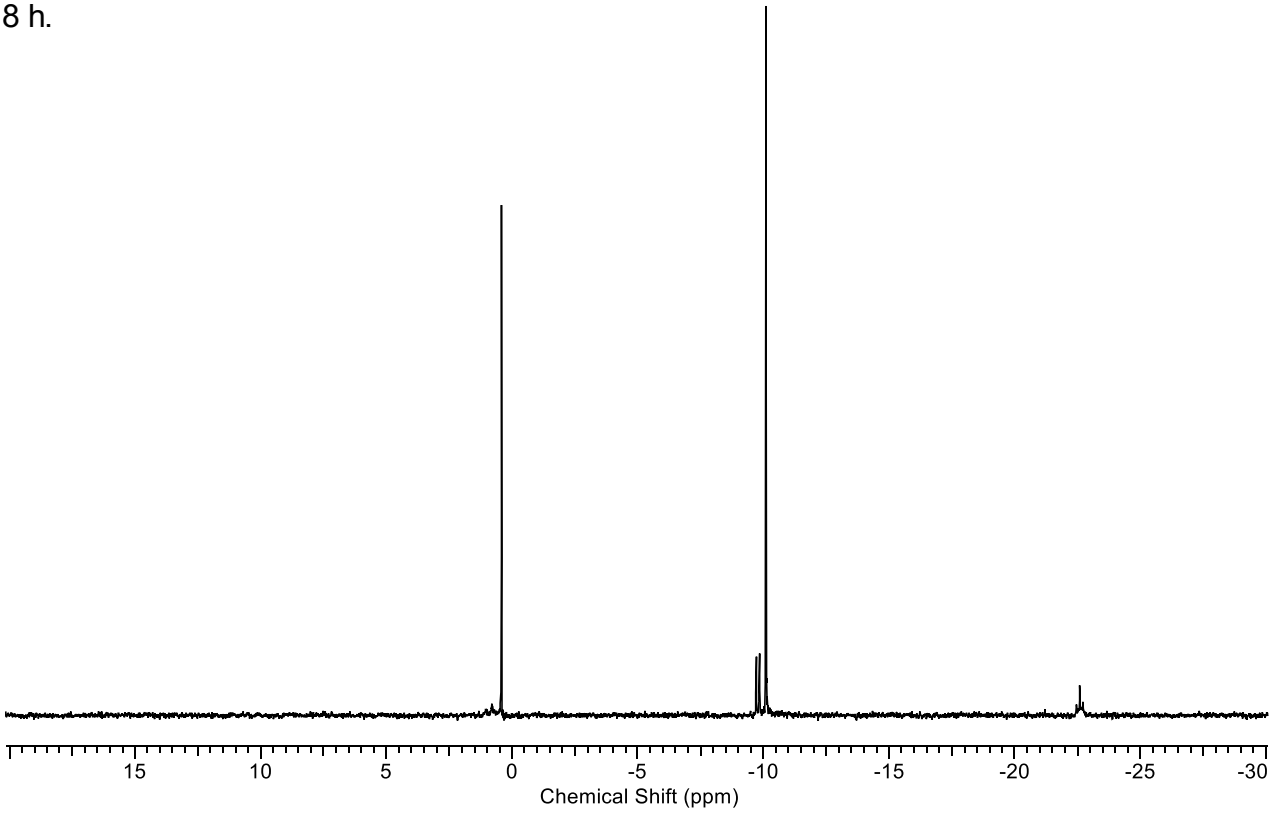


Attempted urea-mediated phosphorylation of hydroxy-aldehydes

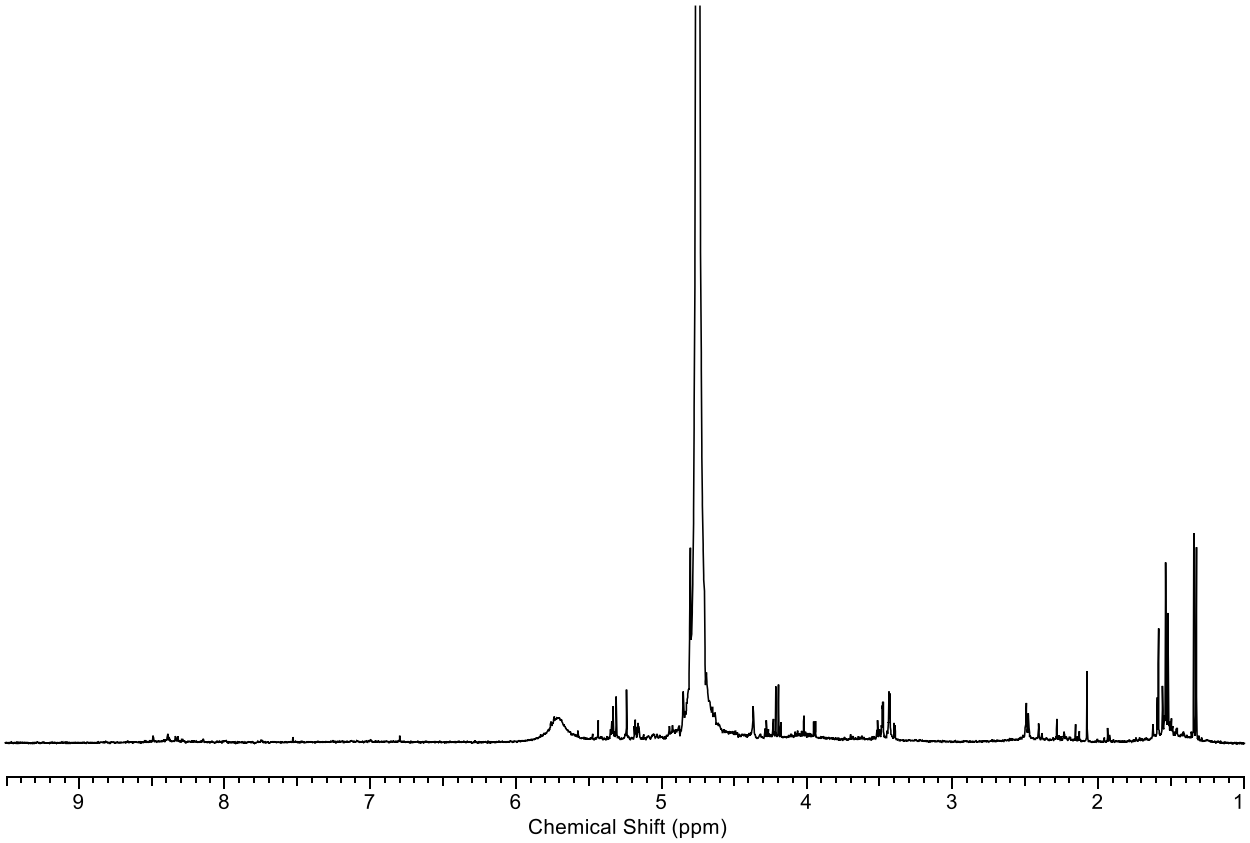
A148: ^{1}H NMR spectrum (400 MHz, {D_2O}, 1.0 – 9.5 ppm) of **A**, the products of the incubation of a thin film of glycolaldehyde (**26**), ammonium dihydrogen phosphate (1 eq.) and urea (10 eq.) at 100 °C for 18 h and **B**, expansion. The three-signal spin-system is due to an un-identified compound, suspected of having a cyclic 5-membered ring structure as shown where the unassigned atoms (X) could each be either O or nitrogen, derived from **26** and urea and based on its similarity to the product of the reaction of **26** (200mM) and cyanamide (**111**, 1 eq.) at pH 7, ambient temperature, for 24 h (shown in blue in spectrum **C**). For a further example of a similar spectrum given by a related heterocycle (endocyclic X = N, exocyclic X = O) see Ritson and Sutherland:[1] δ_H 5.42 (1H, dd, *J* = 6.1, 1.7 Hz); 4.50 (1H dd, *J* = 10.3, 6.1 Hz); 4.18 (1H, dd, *J* = 10.3, 1.7 Hz).



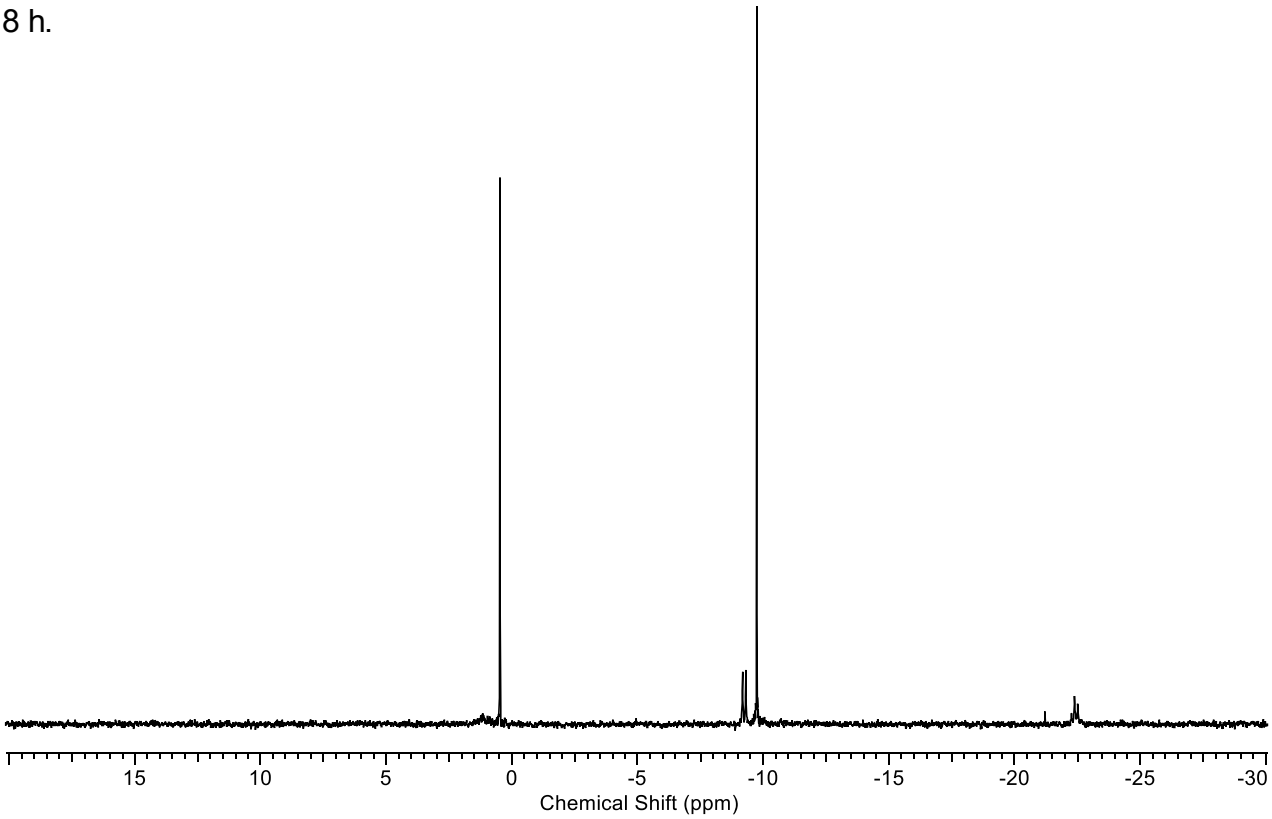
A149: ^{1}H NMR spectrum (162 MHz, {D_2O}, -30 – 20 ppm) of the products of the incubation of a thin film of glycolaldehyde (**26**), ammonium dihydrogen phosphate (1 eq.) and urea (10 eq.) at 100 °C for 18 h.



A150: ^{1}H NMR spectrum (400 MHz, {D_2O}, 1.0 – 9.5 ppm) of the products of the incubation of a thin film of glyceraldehyde (**27**), ammonium dihydrogen phosphate (1 eq.) and urea (10 eq.) at 100 °C for 18 h.

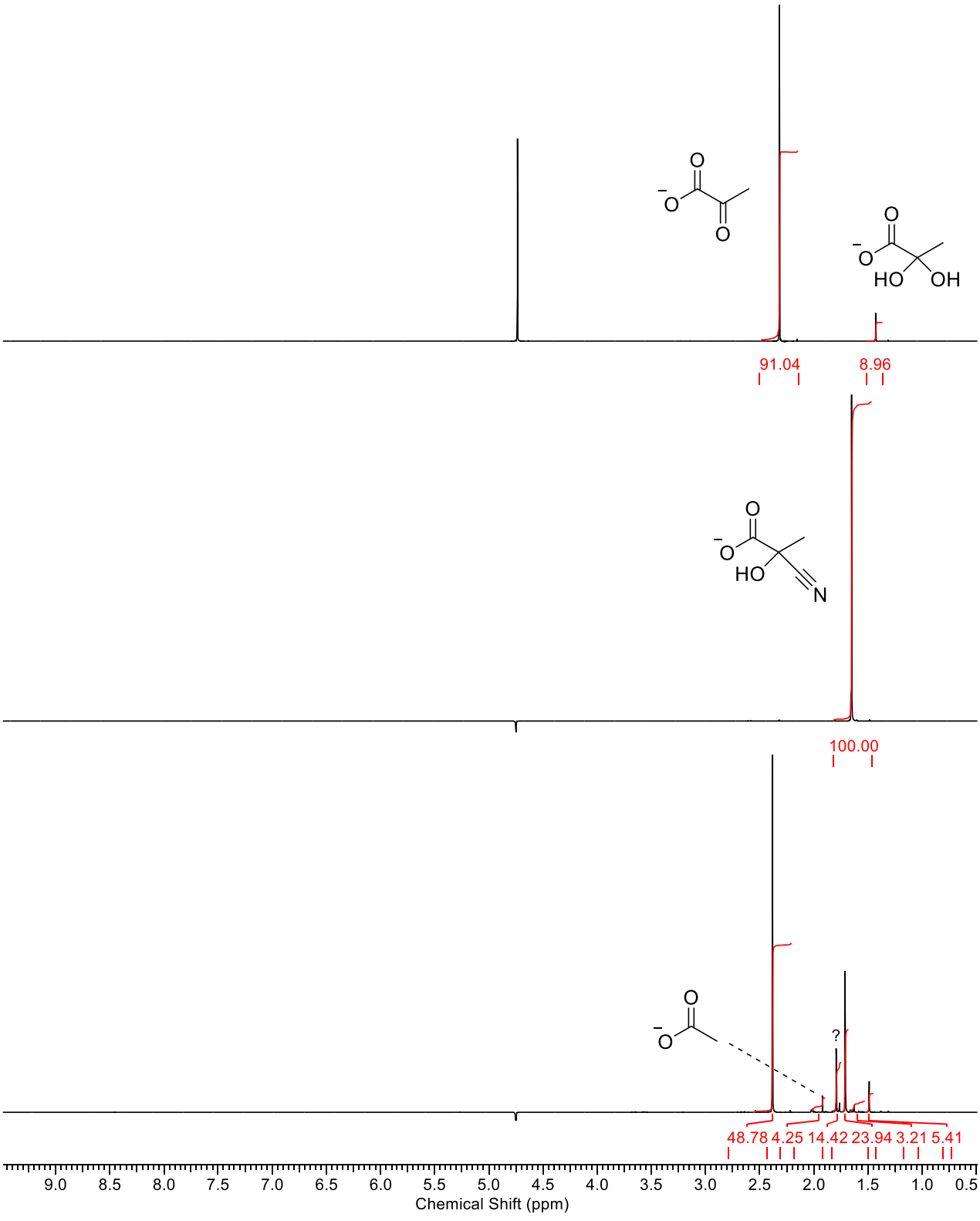


A151: ^{1}H NMR spectrum (162 MHz, {D_2O}, -30 – 20 ppm) of the products of the incubation of a thin film of glyceraldehyde (**27**), ammonium dihydrogen phosphate (1 eq.) and urea (10 eq.) at 100 °C for 18 h.

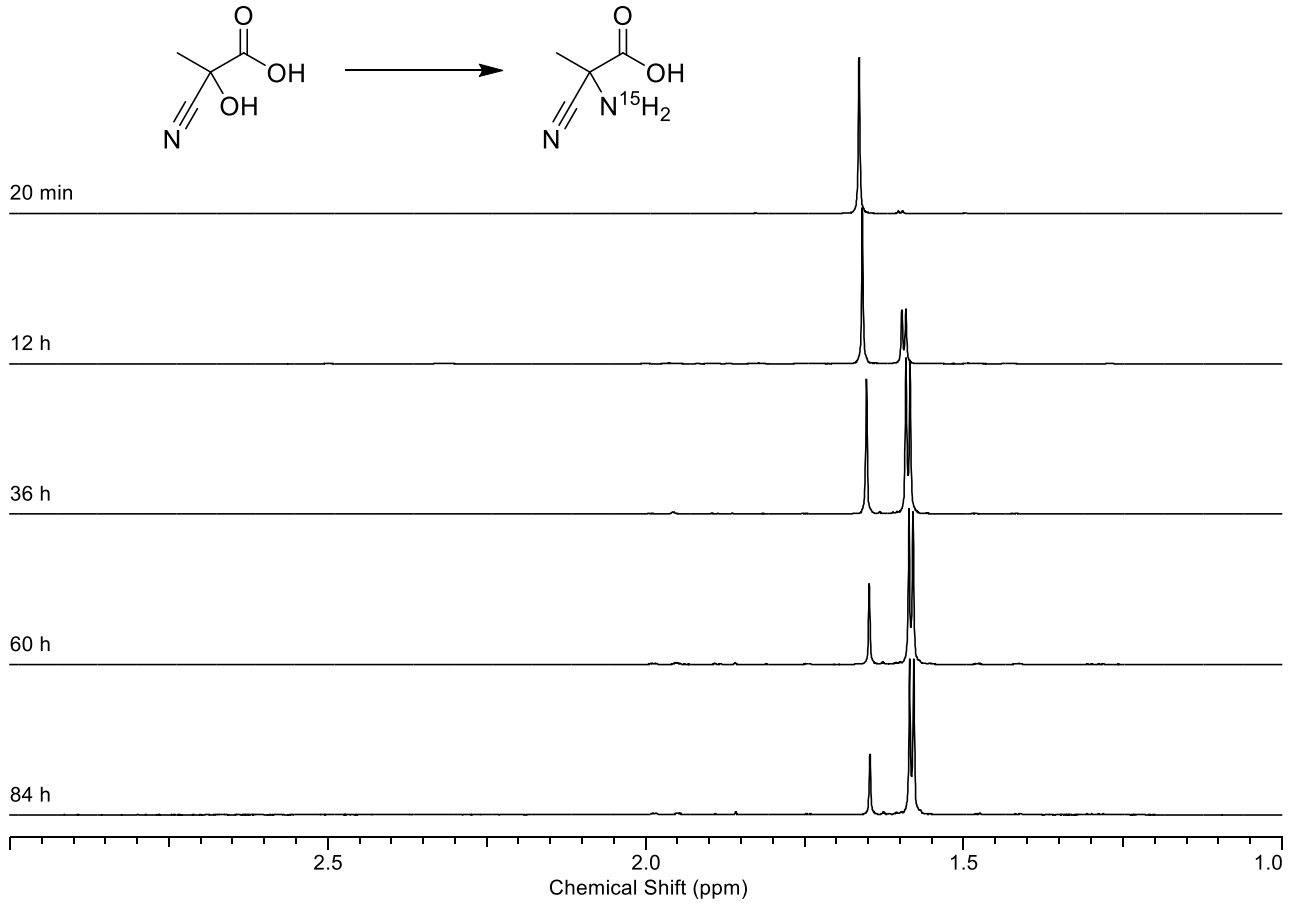


Oxidative decarboxylation experiments

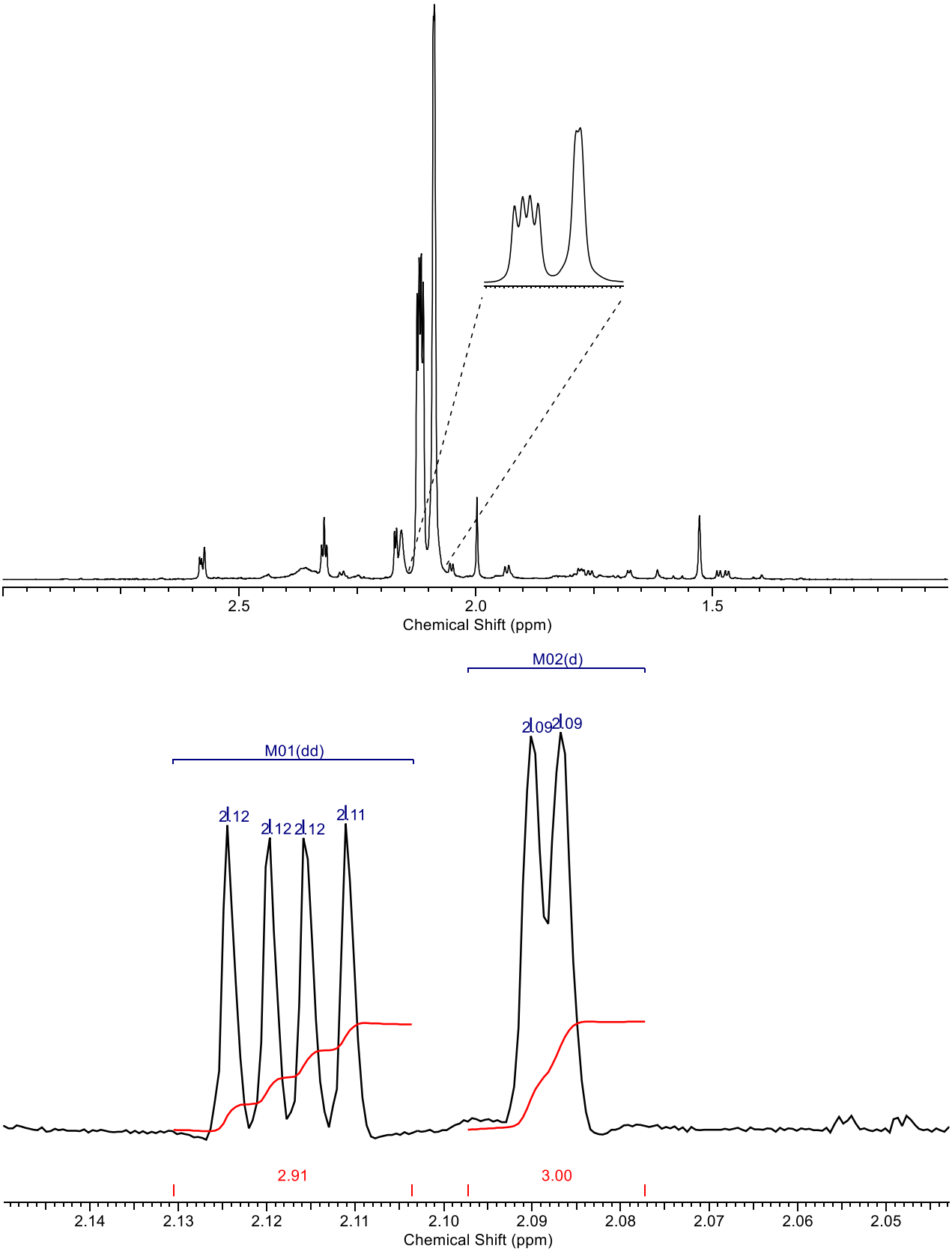
A152: ^{1}H NMR spectra (400 MHz, {H2O/D2O, 9:1}, 0.5 – 9.5 ppm) of **Top**, pyruvate (**64**, 200mM), **Middle**, after incubation with sodium cyanide (1.4 eq.) at pH 6, ambient temperature, 1 h and **Bottom**, after addition of sodium hypochlorite (1 eq.) and incubation at ambient temperature for 30 min.



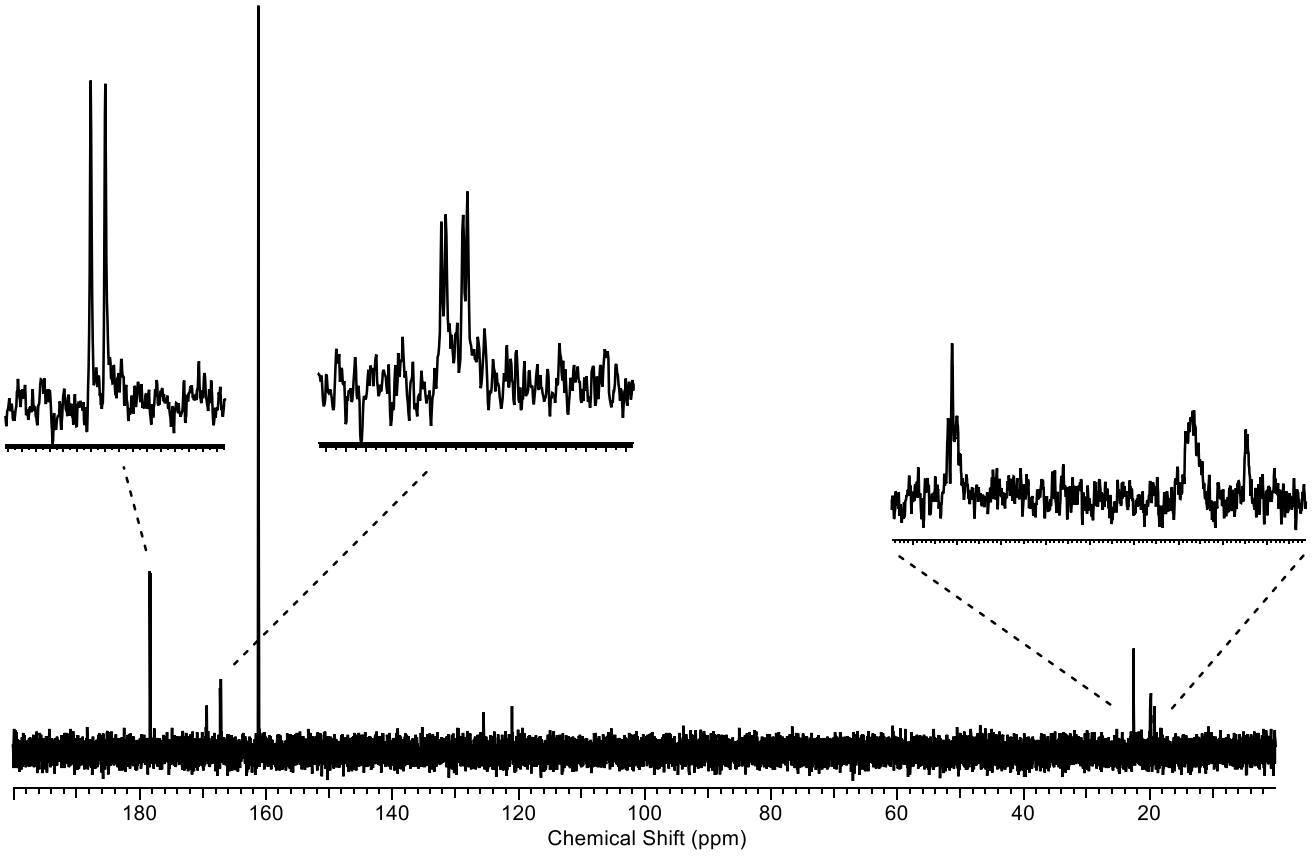
A153: ^{1}H NMR spectra (400 MHz, {H2O/D2O, 9:1}, 1.0 – 3.0 ppm) of pyruvate (**64**, 200mM) with sodium cyanide (280mM) and ^{15}N ammonium chloride (600mM) at pH 9.5, ambient temperature, after various time intervals.



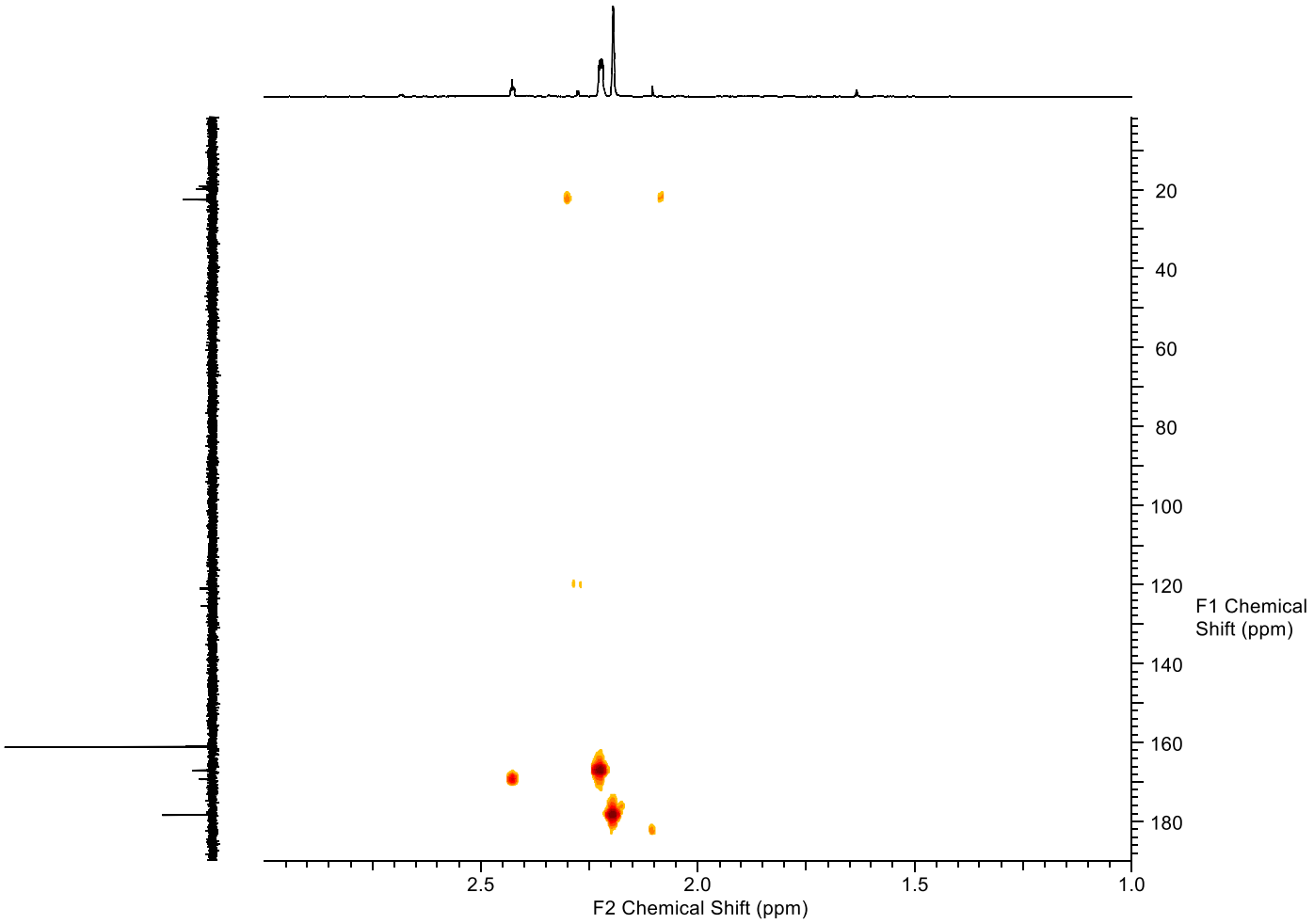
A154: ^{1}H NMR spectrum (400 MHz, {H2O/D2O, 9:1}, 1.0 – 3.0 ppm) of the reaction of pyruvate (**64**, 200mM) with sodium cyanide (280mM) and ^{15}N ammonium chloride (600mM) at pH 9.5, ambient temperature, 84 h, followed by addition of sodium hypochlorite (3 eq.) with expansion overlaid and Fourier transform with Lorentz-Guassian apodisation applied (2.04 – 2.15 ppm) below.



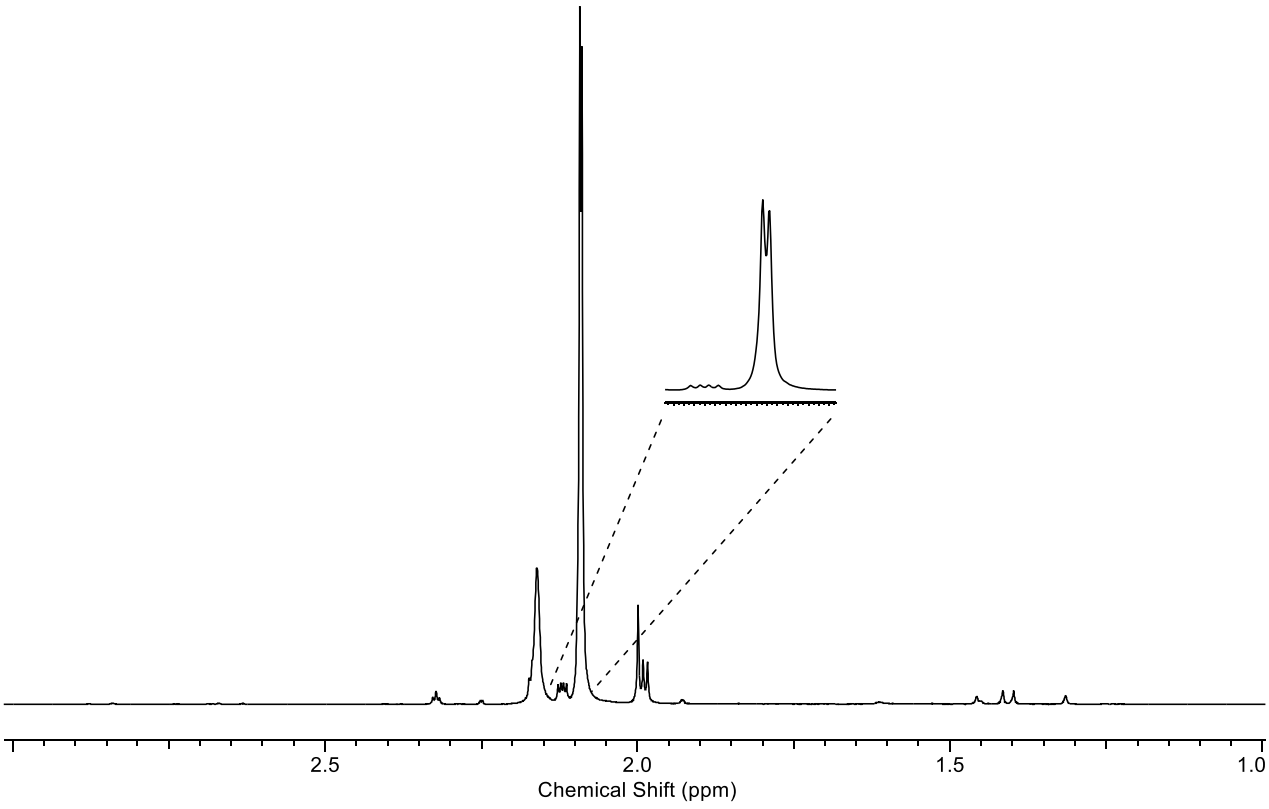
A155: ^{13}C NMR spectrum (151 MHz, {H_2O/D_2O, 9:1}, 0 – 200 ppm) of the reaction of pyruvate (**64**, 200mM) with sodium cyanide (280mM) and ^{15}N ammonium chloride (600mM) at pH 9.5, ambient temperature, 84 h, followed by addition of sodium hypochlorite (3 eq.) with expanded peaks overlaid. The singlet at 161.2 may be due to bicarbonate ion (lit. 161.1),[2] from dissolved CO_2 released by the proposed oxidative decarboxylation mechanism.



A156: ^{1}H-^{13}C HMBC NMR spectrum (600 MHz, {H_2O/D_2O, 9:1}) of the reaction of pyruvate (**64**, 200mM) with sodium cyanide (280mM) and ^{15}N ammonium chloride (600mM) at pH 9.5, ambient temperature, 84 h, followed by addition of sodium hypochlorite (3 eq.).



A157: ^{1}H NMR spectrum (400 MHz, {H2O/D2O, 9:1}, 1.0 – 3.0 ppm) of the reaction of pyruvate (**64**, 200mM) and ^{15}N ammonium chloride (600mM) with sodium hypochlorite (3 eq.) at pH 9.5, ambient temperature with expansion overlaid.



(1) Ritson, D.; Sutherland, J. D. *Nat Chem* **2012**, *4* (11), 895–899.

(2) Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. *J. Org. Chem.* **1997**, *62* (21), 7512–7515.